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A method-comparison study regarding the validity and reliability of the Lactate Plus hand-held lactate meter

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SUMMARY

Article focus

- Determine the validity and reliability of the Nova Biomedical Lactate Plus hand-held lactate meter and quantify any systematic bias.
- Determine the effect of any bias on the determination of lactate threshold
- Determine the effect that blood sampling methods have on validity and reliability

Key messages

- The Lactate Plus portable lactate meter provides valid and reliable measurements of blood lactate concentration.
- The Lactate Plus lactate meter demonstrates a small fixed and proportional bias.
- Sampling directly from the finger does increase the variability in measurement, likely due to the milking of the finger rather than the analyzer itself.

Strengths and limitations

- This study compares the accuracy and variability in measurements under both laboratory and field sampling conditions.
- We used least-product regression analysis to independently quantify fixed and proportional bias rather than Bland-Altman plots or least-squares regression, which lump these bias together or assumes there is no measurement error in the reference method.
- We did not compare either instrument to known lactate standards. This may limit our ability to precisely quantify the accuracy of the hand-held analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. This reduces the likelihood that our reference instrument is inaccurate or non-linear.

ABSTRACT

Objectives: The aims of this study were to: 1) determine the validity and reliability of the Nova Biomedical Lactate Plus hand-held lactate meter, and quantify any fixed or proportional bias, 2) determine the effect of any bias on the determination of the lactate threshold, and 3) determine the effect that blood sampling methods have on validity and reliability. **Design and Participants:** In this method comparison study 15 active men and women performed a graded exercise test to volitional exhaustion. Blood samples were taken via finger prick and collected in micro capillary tubes for analysis by the reference instrument (Yellow Springs Instrument 2300 Glucose and Lactate Analyzer) at the end of each stage. Duplicate samples for the hand-held analyzer were either taken directly from the finger or from the micro capillary tubes. **Primary Outcome Measurements:** Ordinary least products regressions were used to assess validity, reliability, and bias in the hand-held analyzer. **Results:** Though measurements from both instruments were correlated (r=0.91), the differences between instruments had large variability (SD = 1.45mM) when blood was sampled directly from finger. This variability was reduced by ~95% when both instruments measured blood collected in the capillary tubes. As the proportional and fixed bias between instruments was small, there was no difference in estimates of the lactate threshold between instruments. Reliability for the hand-held instrument was strong (r=0.99, $p<0.05$) with no proportional bias (slope=1.02) and small fixed bias (-0.19mM). **Conclusion:** The Lactate Plus analyzer provides accurate and reproducible measurements of blood lactate concentration that can be used to estimate workloads corresponding to blood lactate transitions or absolute lactate concentrations.

INTRODUCTION

Not only is blood lactate accumulation a common measure in the physiological assessment of endurance athletes, but has also been proposed as a measure of metabolic acidosis during fetal examinations.[1] Portable hand-held lactate meters have advantages over bench top models including: 1) their ability to rapidly sample blood lactate concentration ([lactate]), in or outside the laboratory, 2) they require a much smaller sample of blood (0.5 – 0.7μl) than many bench top analyzers, and 3) they can be purchased and operated at a lower cost than many bench top models.

Several studies have attempted to evaluate the validity and reliability of these hand-held analyzers.[1-8]. While the majority of studies report the [lactate] measured using hand-held analyzers is similar to those of various bench top models, the mean difference between the reference and hand-held analyzer can be as much as 1.0 mM. This can represent nearly 10% of the full range of values in some populations. This level of disagreement could be explained by the presence of systematic error, which has gone unexamined in previous studies. Systematic error can result in a proportional bias – where one instrument produces values that are different from those of another instrument by an amount that is proportional to the level of the measured variable. Systematic error can also result in a fixed bias – where one instrument gives values that are different from those of another instrument by a constant amount.[9 10] Therefore, similar mean values between lactate analyzers could occur while the hand-held meter produces low values at lower [lactate] and high values at higher [lactate] or vice-versa. Most previous studies appear to show either a proportional and/or fixed bias.[1 3-8] Because these previous studies have not directly examined these biases it is unclear if they are large enough to affect estimates of various lactate parameters, such as pH or lactate threshold.

Blood sampling techniques may also affect measurement accuracy and reliability. Previous studies have either used intravenous blood drawn directly into a syringe,[4 5 7] or capillary blood from a

finger stick drawn into capillary tubes then mixed as would be done in the laboratory.[3 8] Hand-held meters, however, are designed to sample blood directly from a finger stick for ease of use in the field. When using a finger stick to draw blood it is not uncommon to require "milking" of the finger to get an adequate sample. This may dilute the lactate concentration by increasing interstitial fluid in the sample. Given that duplicate samples are standard practice, this may lead to more variability in measurement values. It would seem important to understand and quantify the effect of differing blood-sampling procedures on the accuracy and reliability of these hand-held lactate meters.

Given the questions that remain regarding the validity and reliability of hand-held lactate meters the specific aims of the present study were: 1) to determine the validity and reliability of the Nova Biomedical Lactate Plus hand-held lactate meter, and quantify any fixed and/or proportional bias, and 2) determine the effect that blood sampling methods have on validity and reliability.

METHODS

Fifteen young (20-36 yr.; mean = 24.5 yrs.) men and women (6 women) participated in the study. All subjects reported at least 90 minutes of moderate to vigorous physical activity each week. All subjects read and signed an informed consent. The Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study. All procedures conformed to the Declaration of Helsinki.

Instruments

To determine the validity of the Lactate Plus hand-held lactate meter we used the YSI 2300 Stat Plus Glucose and Lactate analyzer from Yellow Springs Instruments (Yellow Springs, OH) as our reference instrument. This bench top laboratory analyzer uses a membrane-bound enzyme

electrochemical methodology. L-lactate oxidase is immobilized in a thin membrane placed over an electrochemical probe. The enzyme catalyzes the conversion of L-lactate to pyruvate and hydrogen peroxide, the latter then being oxidized at the platinum anode to measure lactate concentration in whole blood or plasma. A new membrane was used for each data collection session. The analyzer was initially calibrated using a 5mM, 15mM, and 30mM solution. In addition, an automated quality control was performed in triplicate every 45 minutes using a 5mM solution. Blood samples were collected from a finger stick into two heparinized capillary tubes. Blood was then mixed in a micro centrifuge tube. Two 25µl samples were sequentially aspirated and measured by the analyzer.

The Lactate Plus (Nova Biomedical) hand-held lactate meter uses an electrochemical lactate oxidase biosensor to measure lactate concentration in a 0.7 µl sample. As per the manufacturer instructions we used a low (1.0 – 1.6mM) and high (4.0-5.4mM) quality control solution to ensure the lactate meter was operating properly at the beginning of each data collection session. For the first nine participants two blood samples were taken directly from the finger stick as described by the manufacturer. All samples were taken in this order: 1) hand-held directly from finger, 2) capillary tubes for YSI, and 3) hand-held directly from finger. To assess the effect of blood sampling techniques on the accuracy of the hand-held meter blood samples for the last six participants were directly from the same sample used by the YSI 2300.

Graded Exercise

Participants performed a discontinuous graded exercise test (GXT) on a motorized treadmill. Each stage lasted two-minutes with a one-minute blood sampling period between stages. The finger was prepared for sampling just prior to the end of each exercise stage. During the 1-minute blood collection period participants straddled the treadmill belt while blood samples were taken from a finger. After one

minute the participants resumed exercise at a higher speed or grade. The initial speed was 1.55 $m \cdot sec^{-1}$ and 0% grade. The speed was increased either 0.50 or 0.67 $m \cdot sec^{-1}$ for each stage until the participant's heart rate was at least 80% of their age-predicted maximum (220-age). After this point the speed remained constant while grade was increased 2.5% for each stage. Exercise continued until volitional exhaustion.

Data Analysis

Two methods were used to assess validity. First, Bland-Altman plots were constructed to allow the reader to more directly compare our data to that of previous studies since this is the approach typically used. However, because fixed and proportional biases cannot be determined independently from these plots, ordinary least products regression analysis was used. Validity was determined from the correlation coefficient in combination with the presence and degree of bias. The degree of fixed bias was determined from the y-intercept 95% confidence intervals. If the confidence interval for the intercept includes the value of zero, then there is no fixed bias. Proportional bias was determined from the 95% confidence interval for the slope. If the confidence interval for the slope includes the value of 1.0, then there is no proportional bias. Ordinary least products regression gives different slopes and y-intercepts than does least squares regression because error is assumed in both hand-held and bench top analyzers.

Lactate threshold was defined as the point at which blood [lactate] began to increase in a non-linear fashion.[11 12] The threshold was estimated by plotting [lactate] against GXT stage. These graphs were visually inspected to determine the lines of best fit. The equations for each line were set equal to one another and solved for the point of intersection (Figure 1). These equations were also used to calculate the stage that corresponded to an absolute blood [lactate] of 2.5 and 4.0 mM. A t-test for paired data was used to compare means between analyzers. A p-value of <0.05 was considered statistically

significant.

Reliability was determined using ordinary least products regression to quantify the relationship between sequential measurements for both instruments.

RESULTS

Validity

Lactate values during graded exercise ranged from 1.2 to 16.4mM. When both hand-held and bench top blood samples are each taken directly from the finger the mean difference between [lactate] measured by the hand-held lactate meter and the bench top analyzer was small across the full range of lactate values as depicted in Figure 2. While the mean difference between the two instruments was near zero, differences between the instruments had a large variability (SD = 1.45mM). Even though there can be large differences between values measured by the hand-held and bench top analyzer, the paired measurements were highly correlated as shown in Figure 3A. Least products regression indicated a small fixed bias (y-intercept = -0.28mM) between [lactate] measured with the hand-held and bench top analyzers. There was no evidence of a proportional bias (95% CI = 0.94 to 1.15). When the same mixed blood sample was used by both analyzers, the fixed bias was reduced to -0.056mM, while a small proportional bias was evident (slope = 1.08) as shown in Figure 3B.

Regardless of blood sampling approach there was excellent agreement between estimates of the lactate threshold based on lactate values from the hand-held lactate meter compared to those from the bench top analyzer (r = 0.99). Moreover, there was neither a proportional bias (95% CI for slope: 0.843 to 1.083), nor a fixed bias (95% CI for y-intercept: -0.164 to 0.690) in estimates of the lactate threshold from the hand-held analyzer. In addition the stages corresponding to absolute blood lactate values of 2.5 mM ($2.990.41_{NOVA}$ vs. $2.920.44_{YSI}$) and 4.0 mM ($4.640.41_{NOVA}$ vs. $4.610.42_{YSI}$) were not different

between hand-held and bench top values (p = 0.86 for both).

Reliability

The relationship between duplicate measurements of [lactate] by the bench top analyzer was very strong (r=0.99, p<0.05). Ordinary least products regression indicated no proportional bias (slope = 0.99), and a small fixed bias (0.059mM; Figure 4). Ordinary least products regression revealed a small proportional (slope = 1.20) and fixed bias (-0.54mM; Figure 5A) when the two duplicate blood samples for the hand-held analyzer were taken directly from the finger. Thus, the reading from the second sample was typically lower than from the first. However, when two duplicate measurements were taken from the same mixed blood sample, their was no proportional bias (slope = 1.02) and the fixed bias was reduced to -0.19mM).

A total of 242 blood samples were taken using the hand-held analyzer. Twenty-seven of these attempts resulted in error messages (E-4 – insufficient sample). Thus, about 1-in-10 measurement attempts resulted in errors.

DISCUSSION

There were three new findings in our study: 1) The very small proportional bias indicates that the Lactate Plus hand-held lactate meter is a highly linear instrument, 2) multiple blood samples directly from the finger increases measurement error, and 3) the small proportional and fixed bias in the hand-held lactate meter does not affect the ability to determine the lactate threshold.

We chose to use ordinary least products regression to characterize the relation between the Lactate Plus hand-held lactate meter and our reference analyzer. Most studies have employed a

combination of Bland-Altman plots and least squares regression to determine the degree of agreement between various hand-held analyzers and a corresponding reference analyzer.[1-8] The mean difference between analyzers, as determined through Bland-Altman plots, is determined by the interaction of any fixed and proportional bias. Therefore, the mean difference between methods does not solely reflect the accuracy of the device, but in some cases, a loss of linearity. The use of least squares regression to characterize the level of proportional bias, as reflected in the slope of the linear relation, is skewed because all error is assigned to the dependent variable, in this case the hand-held analyzer. The use of least products regression to compare methods avoids both of these issues, allowing independent and more accurate determination of any fixed or proportional bias.[9 10 13]

Numerous studies have compared blood lactate measured with various hand-held lactate meters to several different bench top analyzers.[1 2 5-8] All have reported that these hand-held analyzers produce similar lactate values compared with their bench top counterparts with average differences ranging from -0.8 to 1.0 mM. However, differences of almost 1.0mM can significantly impact the use of absolute [lactate] to characterize training intensity or efficacy. Of the two studies that have tested the Lactate Plus analyzer, only Tanner et al. [8] reported the absolute difference between this hand-held meter and a reference analyzer (-0.8mM). Our data show a much smaller difference between the Lactate Plus hand-held analyzer and the YSI bench top analyzer (fixed bias = -0.056mM). Though not specifically assessed, it does appear that Tanner's reported difference between the hand held and reference analyzer appears to be significantly influenced by a proportional bias (Ref 8, Figures 4 and 5). The fact that our data shows little proportional bias (Figure 3) may account for the greater agreement between analyzers that we observed.

Given that we found a very small proportional bias the estimation of the lactate threshold from [lactate] measured by the Lactate Plus hand-held analyzer agreed very well with those determined from

[lactate] measured by the reference analyzer. Moreover, given the small fixed bias, it was not surprising that the lactate values from the hand-held analyzer provided similar estimates of the workload corresponding to the 2.5 mM and the 4.0 mM absolute lactate concentrations. These data indicate that the Lactate Plus hand-held lactate meter can be used to accurately determine exercise intensities based on blood lactate concentrations.

Duplicate sample readings from the Lactate Plus were strongly related, however there was a small fixed bias, indicating that the values from the second sample were consistently lower than values from the first sample. In addition, there was a very small proportional bias. Both of these biases may be explained by using separate samples collected directly from the finger. The milking of the finger to obtain a blood sample can cause the dilution of the blood sample by interstitial fluid. When we used the same mixed blood sample as the reference analyzer, the proportional bias was eliminated, while the fixed bias was reduced by approximately 65%. Thus, given the high reliability of the Lactate Plus hand-held lactate meter, single samples should prove sufficient. However, we also found that the hand-held analyzer was unable to analyze the blood sample 11% of the time, presumably from an insufficient sample volume. This would require resampling in these cases.

Ridenour et al. advocated for a switch from fetal blood sampling to lactate analysis. [1] However, their data showed that the variability in blood [lactate] accounted for only 46% of the variability in pH. This could be due to the significant proportional bias that is apparent in their data (Ref 1, Figures 1 and 3). However, our analysis shows a fixed and proportional bias that are less than half reported by previous studies relying on Bland-Altman plots and simple comparison of means. [1 4] This suggests the modest correlation between fetal [lactate] and blood pH is best attributed to the independent regulation of blood lactate and pH rather than unreliable measurement of [lactate]. [14 15]

In summary, the Lactate Plus hand-held lactate analyzer is a valid and reliable instrument across

a wide range of blood lactate concentrations. Any proportional or fixed bias in blood lactate concentration is nearly indistinguishable from zero. Therefore, the hand-held analyzer can be used to determine exercise intensities based on absolute or relative blood lactate concentrations. Sampling procedures can have a significant effect on the reliability of the hand-held analyzer, and the hand-held analyzer is prone to technical issues in nearly one out of ten samples.

CONTRIBUTORS

Sarah Drevets collected and analyzed blood samples, and helped with data reduction.

Kathryn Drevets collected and analyzed blood samples, and helped with data reduction.

Micah Alford collected some data and performed statistical analysis.

Amanda Salacinski helped design the study, collected data, and revised the manuscript.

Brian E. Hunt designed the study, collected data, designed statistical analysis, and wrote the manuscript.

COMPETING INTERESTS

None

ETHICS APPROVAL

Ethics approval was provided by the Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study.

DATA SHARING STATEMEMNT

No additional data are available

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Lactate test strips and quality assurance solutions for the Lactate Plus hand-held lactate meter were provided by Lactate.com.

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FIGURE LEGENDS

Figure 1. Lactate threshold determination. Thresholds from YSI data and Lactate Plus data were determined independently.

Figure 2. Bland-Altman plot depicting the level of agreement between lactate concentrations determined by Lactate Plus hand-held analyzer the YSI bench top analyzer.

Figure 3. Ordinary least products regression analysis of the relation between lactate concentrations determined by the Lactate Plus hand-held analyzer and the YSI bench top analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equations and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 4. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the YSI bench top analyzer. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 5. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the Lactate Plus hand-held lactate analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 1

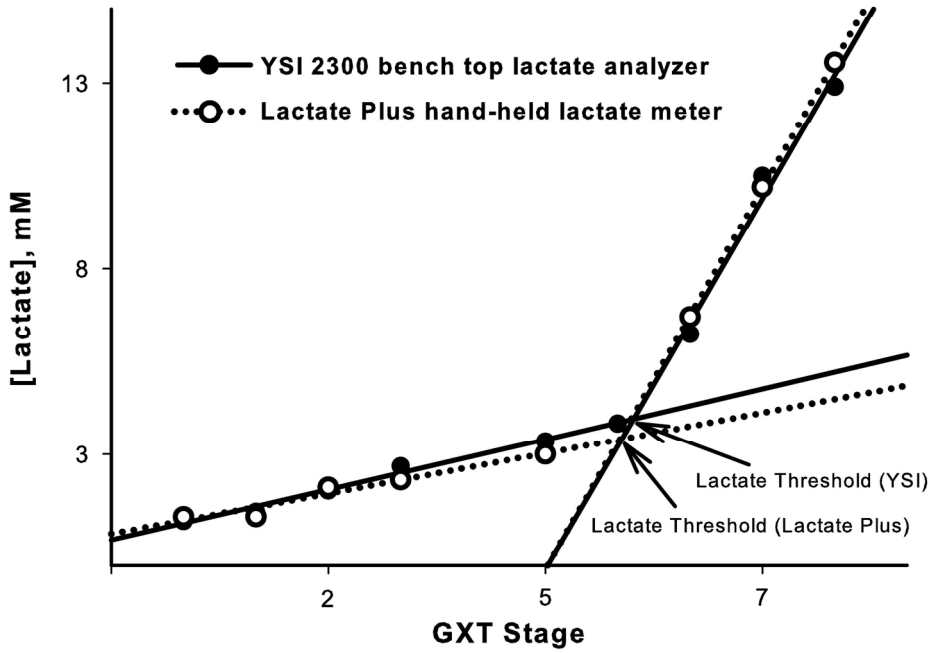

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Figure 2

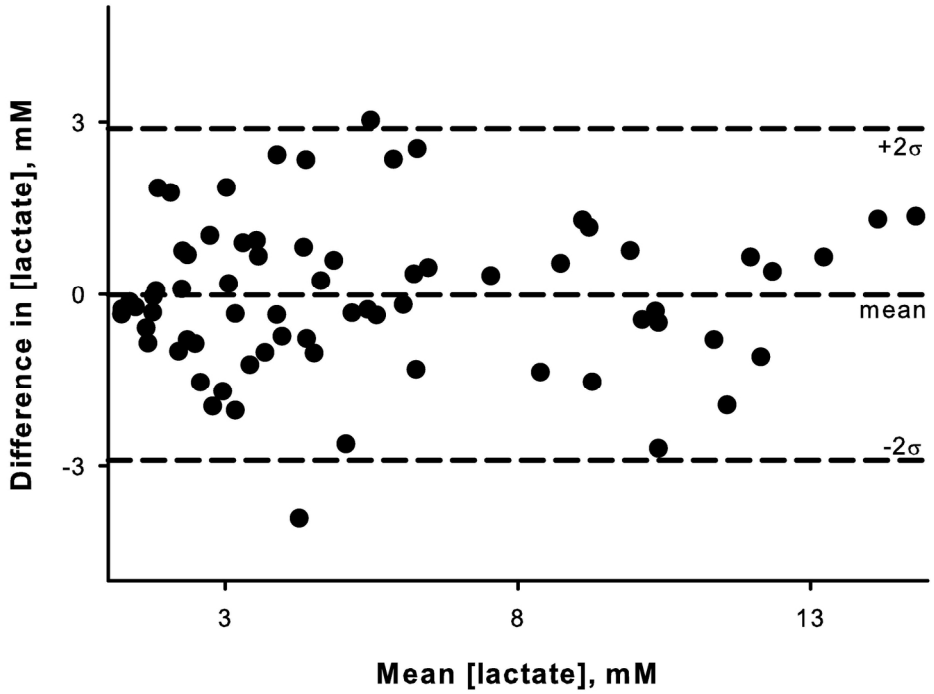

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Figure 3

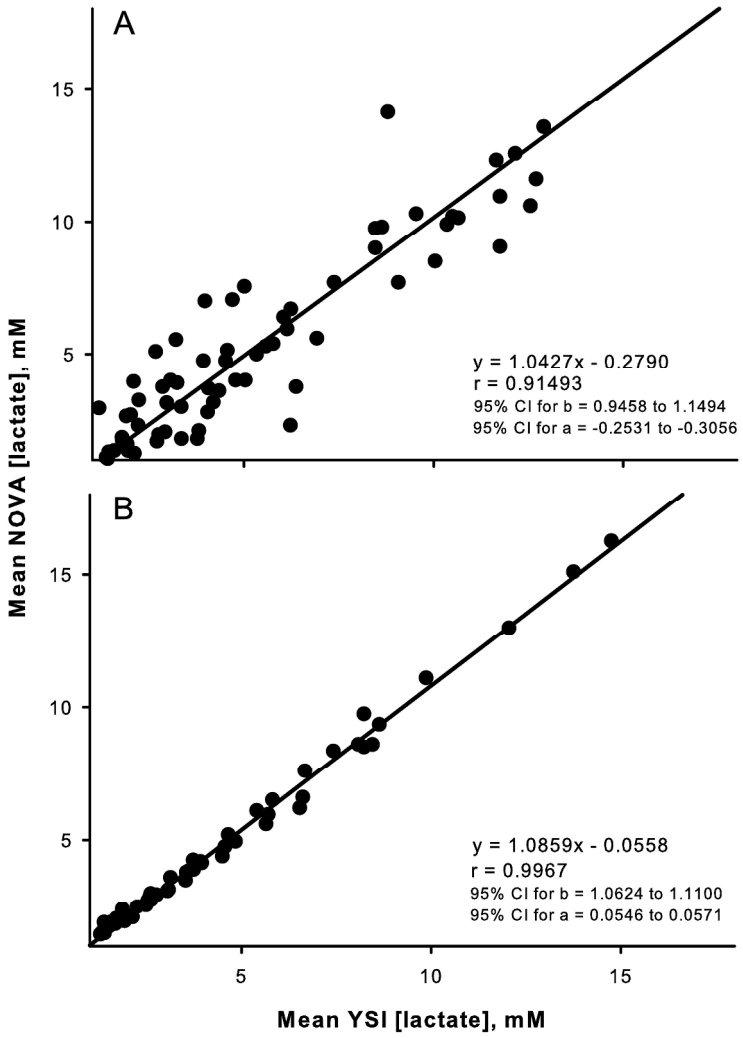

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Figure 4

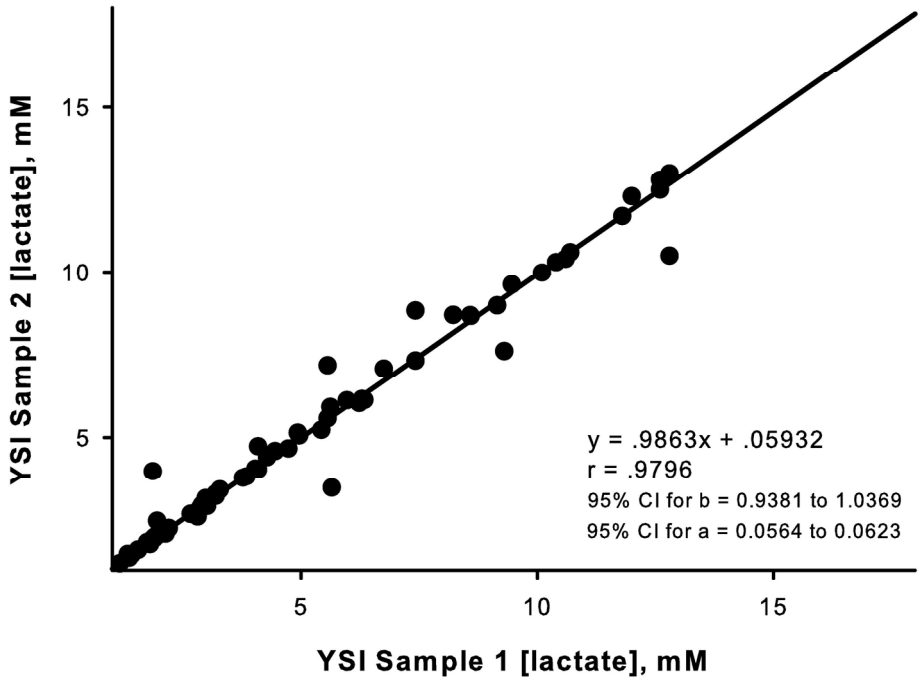

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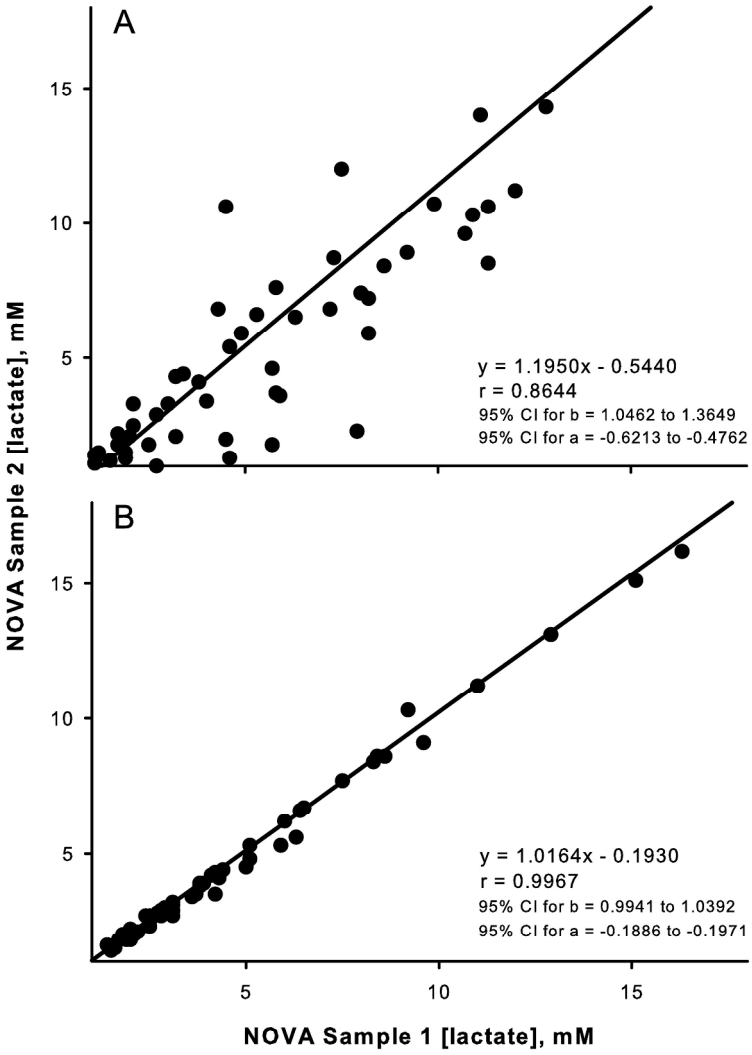
Figure 5



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- Determine the validity and reliability of the Lactate Plus hand-held lactate meter and quantify any systematic bias.
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- Determine the effect that blood sampling methods have on validity and reliability

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- The Lactate Plus portable lactate meter provides valid and reliable measurements of blood lactate concentration.
- The Lactate Plus lactate meter demonstrates a small fixed and proportional bias.
- Sampling directly from the finger does increase the variability in measurement, likely due to the milking of the finger rather than the analyzer itself.

Strengths and limitations

- This study compares the accuracy and variability in measurements under both laboratory and field sampling conditions.
- We used least-product regression analysis to independently quantify fixed and proportional bias rather than Bland-Altman plots or least-squares regression, which lump these bias together or assumes there is no measurement error in the reference method.
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ABSTRACT

Objectives: The aims of this study were to: 1) determine the validity and reliability of the Nova Biomedical Lactate Plus hand-held lactate meter, and quantify any fixed or proportional bias, 2) determine the effect of any bias on the determination of the lactate threshold, and 3) determine the effect that blood sampling methods have on validity and reliability. **Design and Participants:** In this method comparison study 15 active men and women performed a discontinuous graded exercise test to volitional exhaustion on a motorized treadmill. Blood samples were taken via finger prick and collected in micro capillary tubes for analysis by the reference instrument (Yellow Springs Instrument 2300 Glucose and Lactate Analyzer) at the end of each stage. Duplicate samples for the hand-held analyzer were either taken directly from the finger or from the micro capillary tubes. **Primary Outcome Measurements:** Ordinary least products regressions were used to assess validity, reliability, and bias in the hand-held analyzer. Lactate threshold was determined by visual inspection. **Results:** Though measurements from both instruments were correlated (r=0.91), the differences between instruments had large variability (SD = 1.45 $mM \cdot l^{-1}$) when blood was sampled directly from finger. This variability was reduced by ~95% when both instruments measured blood collected in the capillary tubes. As the proportional and fixed bias between instruments was small, there was no difference in estimates of the lactate threshold between instruments. Reliability for the hand-held instrument was strong (r=0.99, $p<0.05$) with no proportional bias (slope=1.02) and small fixed bias (-0.19 $mM \cdot l^{-1}$). **Conclusion:** The Lactate Plus analyzer provides accurate and reproducible measurements of blood lactate concentration that can be used to estimate workloads corresponding to blood lactate transitions or any absolute lactate concentrations.

INTRODUCTION

Not only is blood lactate accumulation a common measure in the physiological assessment of endurance athletes, but has also been proposed as a measure of metabolic acidosis during fetal examinations.[1] Portable hand-held lactate meters have advantages over bench top models including: 1) their ability to rapidly sample blood lactate concentration ([lactate]), in or outside the laboratory, 2) they require a much smaller sample of blood (0.5 – 0.7µl) than many bench top analyzers (25 – 75µl), and 3) they can be purchased and operated at a lower cost than many bench top models.

Several studies have attempted to evaluate the validity and reliability of these hand-held analyzers.[1-8] While the majority of studies report the [lactate] measured using hand-held analyzers is similar to those of various bench top models, the mean difference between the reference and hand-held analyzer can be as much as 1.0 $mM \cdot l^{-1}$. This can represent nearly 10% of the full range of values in some populations. [9] This level of disagreement could be explained by the presence of systematic measurement error, which has gone unexamined in previous studies. Systematic measurement error can result in a proportional bias – where one instrument produces values that are different from those of another instrument by an amount that is proportional to the level of the measured variable. Systematic error can also result in a fixed bias – where one instrument gives values that are different from those of another instrument by a constant amount.[10 11] Therefore, similar mean values between lactate analyzers could occur while the hand-held meter produces low values at lower [lactate] and high values at higher [lactate] or vice-versa. Most previous studies appear to show either a proportional and/or fixed bias.[1 3-8] Because these previous studies have not directly examined these biases it is unclear if they are large enough to affect estimates of various lactate parameters, such as pH or lactate threshold.

Blood sampling techniques may also affect measurement accuracy and reliability. Previous studies have either used intravenous blood drawn directly into a syringe,[4 5 7] or capillary blood from a

finger stick drawn into capillary tubes then mixed as would be done in the laboratory.[3 8] Hand-held meters, however, are designed to sample blood directly from a puncture for ease of use in the field. When using a finger stick to draw blood it is not uncommon to require "milking" of the finger to get an adequate sample. This may dilute the lactate concentration by increasing interstitial fluid in the sample. It would seem important to understand and quantify the effect of differing blood-sampling procedures on the accuracy and reliability of these hand-held lactate meters.

Given the questions that remain regarding the validity and reliability of hand-held lactate meters the specific aims of the present study were: 1) to determine the validity and reliability of the Lactate Plus lactate meter (Nova Biomedical), and quantify any fixed and/or proportional bias, and 2) determine the effect that blood sampling methods have on validity and reliability.

METHODS

Fifteen young (20-36 yr.; mean = 24.5 yr.) men and women (6 women) participated in the study. All subjects reported at least 90 minutes of moderate to vigorous physical activity each week. All subjects read and signed an informed consent. The Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study. All procedures conformed to the Declaration of Helsinki.

Instruments

To determine the validity of the Lactate Plus lactate meter we used the YSI 2300 Stat Plus Glucose and Lactate analyzer from Yellow Springs Instruments (Yellow Springs, OH) as our reference instrument. This bench top laboratory analyzer uses a membrane-bound enzyme electrochemical methodology. L-lactate oxidase is immobilized in a thin membrane placed over an electrochemical

probe. The enzyme catalyzes the conversion of L-lactate to pyruvate and hydrogen peroxide, the latter then being oxidized at the platinum anode to measure lactate concentration in whole blood or plasma. A new membrane was used for each data collection session. The analyzer was initially calibrated using a 5 $mM \cdot l^{-1}$, 15 $mM \cdot l^{-1}$, and 30 $mM \cdot l^{-1}$ solution. In addition, an automated quality control was performed in triplicate every 45 minutes using a 5 $mM \cdot l^{-1}$ solution. Blood samples were collected from a finger stick into two heparinized capillary tubes. Blood was then mixed in a micro centrifuge tube. Two 25µl samples were sequentially aspirated and measured by the analyzer.

The Lactate Plus lactate meter uses an electrochemical lactate oxidase biosensor to measure lactate concentration in a 0.7 µl sample. Following the manufacturer instructions we used a low (1.0 – 1.6 $mM \cdot l^{-1}$) and high (4.0-5.4 $mM \cdot l^{-1}$) quality control solution to ensure the lactate meter was operating properly at the beginning of each data collection session. For the first nine participants three blood samples were taken directly from the finger between each stage of the graded exercise test (GXT). All samples were taken in this order: 1) hand-held directly from finger, 2) capillary tubes for the YSI 2300 from the finger, and 3) a second sample directly from finger using the hand-held meter. To assess the effect of blood sampling techniques on the accuracy of the hand-held meter blood was drawn from the finger into capillary tubes and allocated to both the YSI 2300 and hand-held meter for the last six participants.

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Participants performed a discontinuous graded exercise test (GXT) on a motorized treadmill (Quinton TM65). Each stage lasted two-minutes with a one-minute blood sampling period between stages. The finger was prepared for sampling just prior to the end of each exercise stage. During the 1-minute blood collection period participants straddled the treadmill belt while blood samples were taken

from a finger. After one minute the participants resumed exercise at a higher speed or grade. The initial speed was 1.55 $m \cdot s^{-1}$ and 0% grade. The speed was increased either 0.50 or 0.67 $m \cdot s^{-1}$ for each stage until the participant's heart rate was at least 80% of their age-predicted maximum (220-age). After this point the speed remained constant while grade was increased 2.5% for each stage. Exercise continued until volitional exhaustion.

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Two methods were used to assess validity. First, Bland-Altman plots were constructed to allow the reader to more directly compare our data to that of previous studies since this is the approach typically used. However, because fixed and proportional biases cannot be determined independently from these plots, ordinary least products regression analysis was used. Validity was determined from the correlation coefficient in combination with the presence and degree of bias. The degree of fixed bias was determined from the y-intercept 95% confidence intervals. If the confidence interval for the intercept includes the value of zero, then there is no fixed bias. Proportional bias was determined from the 95% confidence interval for the slope. If the confidence interval for the slope includes the value of 1.0, then there is no proportional bias. Ordinary least products regression gives different slopes and y-intercepts than does least squares regression because error is assumed in both hand-held and bench top analyzers.[10 11]

Lactate threshold was defined as the point at which blood [lactate] began to increase in a non-linear fashion.[12 13] The threshold was estimated by plotting [lactate] against GXT stage. These graphs were visually inspected to determine the lines of best fit by the two evaluators. The equations for each line were set equal to one another and solved for the point of intersection (Figure 1). The values from each evaluator were averaged.[14] These equations were also used to calculate the stage that corresponded

to an absolute blood [lactate] of 2.5 and 4.0 $mM \cdot l^{-1}$. A t-test for paired data was used to compare means between analyzers. A p-value of <0.05 was considered statistically significant.

Reliability was determined using ordinary least products regression to quantify the relationship between sequential measurements for both instruments.

RESULTS

Validity

Lactate values during graded exercise ranged from 1.2 to 16.4 $mM \cdot l^{-1}$. When both hand-held and bench top blood samples are each taken directly from the finger the mean difference between [lactate] measured by the hand-held lactate meter and the bench top analyzer was small across the full range of lactate values as depicted in Figure 2. While the mean difference between the two instruments was near zero, differences between the instruments had a large variability (SD = 1.45 $mM \cdot l^{-1}$). Even though there can be large differences between values measured by the hand-held and bench top analyzer, the paired measurements were highly correlated as shown in Figure 3A. Least products regression indicated a small fixed bias (y-intercept = -0.28 $mM \cdot l^{-1}$) between [lactate] measured with the hand-held and bench top analyzers. There was no evidence of a proportional bias (95% CI = 0.94 to 1.15). When the same mixed blood sample was used by both analyzers, the fixed bias was reduced to -0.056 $mM \cdot l^{-1}$, while a small proportional bias was evident (slope = 1.08) as shown in Figure 3B.

Regardless of blood sampling approach there was excellent agreement between estimates of the lactate threshold based on lactate values from the hand-held lactate meter compared to those from the bench top analyzer (r = 0.97). Moreover, there was neither a proportional bias (95% CI for slope: 0.910 to 1.098), nor a fixed bias (95% CI for y-intercept: -0.396 to 0.325) in estimates of the lactate threshold from the hand-held analyzer. In addition the stages corresponding to absolute blood lactate values of 2.5

$mM \cdot l^{-1}$ (2.99_{NOVA} vs. 2.92_{YSI}) and 4.0 $mM \cdot l^{-1}$ (4.64_{NOVA} vs. 4.61_{YSI}) were not different between hand-held and bench top values ($p = 0.86$ for both).

Reliability

The relationship between duplicate measurements of [lactate] by the bench top analyzer was very strong ($r=0.99$, $p<0.05$). Ordinary least products regression indicated no proportional bias (slope = 0.99), and a small fixed bias (0.059 $mM \cdot l^{-1}$; Figure 4). Ordinary least products regression revealed a small proportional (slope = 1.20) and fixed bias (-0.54 $mM \cdot l^{-1}$; Figure 5A) when the two duplicate blood samples for the hand-held analyzer were taken directly from the finger. Thus, the reading from the second sample was typically lower than from the first. However, when two duplicate measurements were taken from the same mixed blood sample, their was no proportional bias (slope = 1.02) and the fixed bias was reduced to -0.19 $mM \cdot l^{-1}$).

A total of 242 blood samples were taken using the hand-held analyzer. Twenty-seven of these attempts resulted in error messages (E-4 – insufficient sample). Thus, about 1-in-10 measurement attempts resulted in errors.

DISCUSSION

There were three new findings in our study: 1) The very small proportional bias indicates that the Lactate Plus lactate meter is a highly linear instrument, 2) multiple blood samples directly from the finger increases measurement error, and 3) the small proportional and fixed bias in the hand-held lactate meter does not affect the ability to determine the lactate threshold.

We chose to use ordinary least products regression to characterize the relation between the

Lactate Plus lactate meter and our reference analyzer. Most studies have employed a combination of Bland-Altman plots and least squares regression to determine the degree of agreement between various hand-held analyzers and a corresponding reference analyzer.[1-8] The mean difference between analyzers, as determined through Bland-Altman plots, is determined by the interaction of any fixed and proportional bias. Therefore, the mean difference between methods does not solely reflect the accuracy of the device, but in some cases, a loss of linearity. The use of least squares regression to characterize the level of proportional bias, as reflected in the slope of the linear relation, is skewed because all error is assigned to the dependent variable, in this case the hand-held analyzer. The use of least products regression to compare methods avoids both of these issues, allowing independent and more accurate determination of any fixed or proportional bias.[10 11 15]

Numerous studies have compared blood lactate measured with various hand-held lactate meters to several different bench top analyzers.[1 2 5-8] All have reported that these hand-held analyzers produce similar lactate values compared with their bench top counterparts with average differences ranging from -0.8 to 1.0 $mM \cdot l^{-1}$. However, differences of almost 1.0 $mM \cdot l^{-1}$ can significantly impact the use of absolute [lactate] to characterize training intensity or efficacy. Weltman et al. reported that women who trained at an intensity corresponding to about 2.5 $mM \cdot l^{-1}$ showed greater improvement in blood lactate parameters, but less of an improvement in VO2max than did women training at their lactate threshold. [16] If true, then an error in the measurement of blood lactate concentration could lead to suboptimal improvements in either lactate parameters or VO2max. Of the two studies that have tested the Lactate Plus lactate meter, only Tanner et al. [8] reported the absolute difference between this hand-held meter and a reference analyzer (-0.8 $mM \cdot l^{-1}$). Our data show a much smaller difference between the Lactate Plus lactate meter and the YSI bench top analyzer (fixed bias = -0.056 $mM \cdot l^{-1}$). Though not specifically assessed, it does appear that Tanner's reported difference between the hand held and reference analyzer

is significantly influenced by a proportional bias (Ref 8, Figures 4 and 5). The fact that our data shows little proportional bias (Figure 3) may account for the greater agreement between analyzers that we observed. It is possible that if Tanner had been able to independently determine the proportional and fixed bias, their analysis may have revealed a small bias similar to ours. Differences in reference instruments would not likely explain the greater measurement error reported by Tanner, given that their instrument undergoes a 3-point calibration and 2-point calibration check every few hours, similar to our reference instrument.

Given that we found a very small proportional bias the estimation of the lactate threshold from [lactate] measured by the Lactate Plus lactate meter agreed very well with those determined from [lactate] measured by the reference analyzer. Moreover, given the small fixed bias, it was not surprising that the lactate values from the hand-held analyzer provided similar estimates of the workload corresponding to the 2.5 $mM \cdot l^{-1}$ and the 4.0 $mM \cdot l^{-1}$ absolute lactate concentrations. These data indicate that the Lactate Plus lactate meter can be used to accurately determine exercise intensities based on blood lactate concentrations.

Duplicate sample readings from the Lactate Plus lactate meter were strongly related, however there was a small fixed bias, indicating that the values from the second sample were consistently lower than values from the first sample. In addition, there was a very small proportional bias. Both of these biases may be explained by using separate samples collected directly from the finger. The milking of the finger to obtain a blood sample can cause the dilution of the blood sample by interstitial fluid. The manufacturer warns the user against vigorous squeezing of the finger to obtain a blood drop. The use of a vasodilating cream may resolve this issue. When we used the same mixed blood sample as the reference analyzer, the proportional bias was eliminated, while the fixed bias was reduced by approximately 65%.

We also found that the hand-held analyzer was unable to analyze the blood sample 11% of the time, presumably from an insufficient sample volume. This was surprising given that the Lactate Plus lactate meter provides an audible signal to indicate when the test strip has a sufficient volume of blood for analysis. Our experience has shown that anticipating the filling of the test strip can result in both the audible signal and an error. However, even when great care is taken, one can still get an audible full signal and the error message.

Ridenour et al. advocated for a switch from fetal blood sampling to lactate analysis. [1] However, their data showed that the variability in blood [lactate] accounted for only 46% of the variability in pH. This could be due to the significant proportional bias that is apparent in their data (Ref 1, Figures 1 and 3). However, our analysis shows a fixed and proportional bias that are less than half reported by previous studies relying on Bland-Altman plots and simple comparison of means. [1 4] This suggests the modest correlation between fetal [lactate] and blood pH is best attributed to the independent regulation of blood lactate and pH rather than unreliable measurement of [lactate]. [17 18]

We did not compare the Lactate Plus lactate meter to known standards. This limits the precision with which we can quantify the accuracy of the hand-held analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. Our analysis assumes measurement error in both the hand-held and reference instrument. Thus it is likely that by comparing the Lactate Plus lactate meter directly to known lactate standards, our fixed bias would be reduced.

In summary, the Lactate Plus lactate meter is a valid and reliable instrument across a wide range of blood lactate concentrations. Any proportional or fixed bias in blood lactate concentration is nearly indistinguishable from zero. Therefore, the hand-held analyzer can be used to determine exercise intensities based on absolute or relative blood lactate concentrations. Sampling procedures can have a significant effect on the reliability of the hand-held analyzer, and the hand-held analyzer is prone to

technical issues in nearly one out of ten samples.

CONTRIBUTORS

Sarah Drevets collected and analyzed blood samples, and helped with data reduction.

Kathryn Drevets collected and analyzed blood samples, and helped with data reduction.

Micah Alford collected some data and performed statistical analysis.

Amanda Salacinski helped design the study, collected data, and revised the manuscript.

Brian E. Hunt designed the study, collected data, designed statistical analysis, and wrote the manuscript.

COMPETING INTERESTS

None

ETHICS APPROVAL

Ethics approval was provided by the Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study.

DATA SHARING STATEMEMNT

No additional data are available

ACKNOWLDGMENTS

Lactate test strips and quality assurance solutions for the Lactate Plus lactate meter were provided by Lactate.com.

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FIGURE LEGENDS

Figure 1. Determination of the Lactate threshold by visual inspection. Shown are data from a representative study participant and the lines of best fit that were determined independently for data from the YSI 2300 lactate analyzer and the Lactate Plus lactate meter.

Figure 2. Bland-Altman plot depicting the level of agreement between lactate concentrations determined by Lactate Plus hand-held analyzer the YSI bench top analyzer.

Figure 3. Ordinary least products regression analysis of the relation between lactate concentrations determined by the Lactate Plus hand-held analyzer and the YSI bench top analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equations and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 4. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the YSI bench top analyzer. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 5. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the Lactate Plus hand-held lactate analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

A method-comparison study regarding the validity and reliability of the Lactate Plus hand-held lactate meter

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SUMMARY

Article focus

- Determine the validity and reliability of the Lactate Plus hand-held lactate meter and quantify any systematic bias.
- Determine the effect of any bias on the determination of lactate threshold
- Determine the effect that blood sampling methods have on validity and reliability

Key messages

- The Lactate Plus portable lactate meter provides valid and reliable measurements of blood lactate concentration.
- The Lactate Plus lactate meter demonstrates a small fixed and proportional bias.
- Sampling directly from the finger does increase the variability in measurement, likely due to the milking of the finger rather than the analyzer itself.

Strengths and limitations

- This study compares the accuracy and variability in measurements under both laboratory and field sampling conditions.
- We used least-product regression analysis to independently quantify fixed and proportional bias rather than Bland-Altman plots or least-squares regression, which lump these bias together or assumes there is no measurement error in the reference method.
- We did not compare either instrument to known lactate standards. This may limit our ability to precisely quantify the accuracy of the hand-held analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. This reduces the likelihood that our reference instrument is inaccurate or non-linear.

ABSTRACT

Objectives: The aims of this study were to: 1) determine the validity and reliability of the Nova Biomedical Lactate Plus hand-held lactate meter, and quantify any fixed or proportional bias, 2) determine the effect of any bias on the determination of the lactate threshold, and 3) determine the effect that blood sampling methods have on validity and reliability. **Design and Participants:** In this method comparison study 15 active men and women performed a discontinuous graded exercise test to volitional exhaustion on a motorized treadmill. Blood samples were taken via finger prick and collected in micro capillary tubes for analysis by the reference instrument (Yellow Springs Instrument 2300 Glucose and Lactate Analyzer) at the end of each stage. Duplicate samples for the hand-held analyzer were either taken directly from the finger or from the micro capillary tubes. **Primary Outcome Measurements:** Ordinary least products regressions were used to assess validity, reliability, and bias in the hand-held analyzer. Lactate threshold was determined by visual inspection. **Results:** Though measurements from both instruments were correlated (r=0.91), the differences between instruments had large variability (SD = 1.45 $mM \cdot l^{-1}$) when blood was sampled directly from finger. This variability was reduced by ~95% when both instruments measured blood collected in the capillary tubes. As the proportional and fixed bias between instruments was small, there was no difference in estimates of the lactate threshold between instruments. Reliability for the hand-held instrument was strong (r=0.99, $p<0.05$) with no proportional bias (slope=1.02) and small fixed bias (-0.19 $mM \cdot l^{-1}$). **Conclusion:** The Lactate Plus analyzer provides accurate and reproducible measurements of blood lactate concentration that can be used to estimate workloads corresponding to blood lactate transitions or any absolute lactate concentrations.

INTRODUCTION

Not only is blood lactate accumulation a common measure in the physiological assessment of endurance athletes, but has also been proposed as a measure of metabolic acidosis during fetal examinations.[1] Portable hand-held lactate meters have advantages over bench top models including: 1) their ability to rapidly sample blood lactate concentration ([lactate]), in or outside the laboratory, 2) they require a much smaller sample of blood (0.5 – 0.7µl) than many bench top analyzers (25 – 75µl), and 3) they can be purchased and operated at a lower cost than many bench top models.

Several studies have attempted to evaluate the validity and reliability of these hand-held analyzers.[1-8] While the majority of studies report the [lactate] measured using hand-held analyzers is similar to those of various bench top models, the mean difference between the reference and hand-held analyzer can be as much as 1.0 $mM \cdot l^{-1}$. This can represent nearly 10% of the full range of values in some populations.[9] This level of disagreement could be explained by the presence of systematic measurement error, which has gone unexamined in previous studies. Systematic measurement error can result in a proportional bias – where one instrument produces values that are different from those of another instrument by an amount that is proportional to the level of the measured variable. Systematic error can also result in a fixed bias – where one instrument gives values that are different from those of another instrument by a constant amount.[10,11] Therefore, similar mean values between lactate analyzers could occur while the hand-held meter produces low values at lower [lactate] and high values at higher [lactate] or vice-versa. Most previous studies appear to show either a proportional and/or fixed bias.[1,3-8] Because these previous studies have not directly examined these biases it is unclear if they are large enough to affect estimates of various lactate parameters, such as pH or lactate threshold.

Blood sampling techniques may also affect measurement accuracy and reliability. Previous studies have either used intravenous blood drawn directly into a syringe,[4,5,7] or capillary blood from a

finger stick drawn into capillary tubes then mixed as would be done in the laboratory.[3 8] Hand-held meters, however, are designed to sample blood directly from a puncture for ease of use in the field. When using a finger stick to draw blood it is not uncommon to require "milking" of the finger to get an adequate sample. This may dilute the lactate concentration by increasing interstitial fluid in the sample. It would seem important to understand and quantify the effect of differing blood-sampling procedures on the accuracy and reliability of these hand-held lactate meters.

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Field Co

to an absolute blood [lactate] of 2.5 and 4.0 $mM \cdot l^{-1}$. A t-test for paired data was used to compare means between analyzers. A p-value of <0.05 was considered statistically significant.

Reliability was determined using ordinary least products regression to quantify the relationship between sequential measurements for both instruments.

RESULTS

Validity

Lactate values during graded exercise ranged from 1.2 to 16.4 $mM \cdot l^{-1}$. When both hand-held and bench top blood samples are each taken directly from the finger the mean difference between [lactate] measured by the hand-held lactate meter and the bench top analyzer was small across the full range of lactate values as depicted in Figure 2. While the mean difference between the two instruments was near zero, differences between the instruments had a large variability (SD = 1.45 $mM \cdot l^{-1}$). Even though there can be large differences between values measured by the hand-held and bench top analyzer, the paired measurements were highly correlated as shown in Figure 3A. Least products regression indicated a small fixed bias (y-intercept = -0.28 $mM \cdot l^{-1}$) between [lactate] measured with the hand-held and bench top analyzers. There was no evidence of a proportional bias (95% CI = 0.94 to 1.15). When the same mixed blood sample was used by both analyzers, the fixed bias was reduced to -0.056 $mM \cdot l^{-1}$, while a small proportional bias was evident (slope = 1.08) as shown in Figure 3B.

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$mM \cdot l^{-1}$ (2.99_{NOVA} vs. 2.92_{YSI}) and 4.0 $mM \cdot l^{-1}$ (4.64_{NOVA} vs. 4.61_{YSI}) were not different between hand-held and bench top values (p = 0.86 for both).

Reliability

The relationship between duplicate measurements of [lactate] by the bench top analyzer was very strong (r=0.99, p<0.05). Ordinary least products regression indicated no proportional bias (slope = 0.99), and a small fixed bias (0.059 $mM \cdot l^{-1}$; Figure 4). Ordinary least products regression revealed a small proportional (slope = 1.20) and fixed bias (-0.54 $mM \cdot l^{-1}$; Figure 5A) when the two duplicate blood samples for the hand-held analyzer were taken directly from the finger. Thus, the reading from the second sample was typically lower than from the first. However, when two duplicate measurements were taken from the same mixed blood sample, their was no proportional bias (slope = 1.02) and the fixed bias was reduced to -0.19 $mM \cdot l^{-1}$).

A total of 242 blood samples were taken using the hand-held analyzer. Twenty-seven of these attempts resulted in error messages (E-4 – insufficient sample). Thus, about 1-in-10 measurement attempts resulted in errors.

DISCUSSION

There were three new findings in our study: 1) The very small proportional bias indicates that the Lactate Plus lactate meter is a highly linear instrument, 2) multiple blood samples directly from the finger increases measurement error, and 3) the small proportional and fixed bias in the hand-held lactate meter does not affect the ability to determine the lactate threshold.

We chose to use ordinary least products regression to characterize the relation between the

Lactate Plus lactate meter and our reference analyzer. Most studies have employed a combination of Bland-Altman plots and least squares regression to determine the degree of agreement between various hand-held analyzers and a corresponding reference analyzer.[1-8] The mean difference between analyzers, as determined through Bland-Altman plots, is determined by the interaction of any fixed and proportional bias. Therefore, the mean difference between methods does not solely reflect the accuracy of the device, but in some cases, a loss of linearity. The use of least squares regression to characterize the level of proportional bias, as reflected in the slope of the linear relation, is skewed because all error is assigned to the dependent variable, in this case the hand-held analyzer. The use of least products regression to compare methods avoids both of these issues, allowing independent and more accurate determination of any fixed or proportional bias.[10 11 15]

Numerous studies have compared blood lactate measured with various hand-held lactate meters to several different bench top analyzers.[1 2 5-8] All have reported that these hand-held analyzers produce similar lactate values compared with their bench top counterparts with average differences ranging from -0.8 to 1.0 $mM \cdot l^{-1}$. However, differences of almost 1.0 $mM \cdot l^{-1}$ can significantly impact the use of absolute [lactate] to characterize training intensity or efficacy. Weltman et al. reported that women who trained at an intensity corresponding to about 2.5 $mM \cdot l^{-1}$ showed greater improvement in blood lactate parameters, but less of an improvement in VO2max than did women training at their lactate threshold. [16] If true, then an error in the measurement of blood lactate concentration could lead to suboptimal improvements in either lactate parameters or VO2max. Of the two studies that have tested the Lactate Plus lactate meter, only Tanner et al. [8] reported the absolute difference between this hand-held meter and a reference analyzer (-0.8 $mM \cdot l^{-1}$). Our data show a much smaller difference between the Lactate Plus lactate meter and the YSI bench top analyzer (fixed bias = -0.056 $mM \cdot l^{-1}$). Though not specifically assessed, it does appear that Tanner's reported difference between the hand held and reference analyzer

is significantly influenced by a proportional bias (Ref 8, Figures 4 and 5). The fact that our data shows little proportional bias (Figure 3) may account for the greater agreement between analyzers that we observed. It is possible that if Tanner had been able to independently determine the proportional and fixed bias, their analysis may have revealed a small bias similar to ours. Differences in reference instruments would not likely explain the greater measurement error reported by Tanner, given that their instrument undergoes a 3-point calibration and 2-point calibration check every few hours, similar to our reference instrument.

Given that we found a very small proportional bias the estimation of the lactate threshold from [lactate] measured by the Lactate Plus lactate meter agreed very well with those determined from [lactate] measured by the reference analyzer. Moreover, given the small fixed bias, it was not surprising that the lactate values from the hand-held analyzer provided similar estimates of the workload corresponding to the 2.5 $mM \cdot l^{-1}$ and the 4.0 $mM \cdot l^{-1}$ absolute lactate concentrations. These data indicate that the Lactate Plus lactate meter can be used to accurately determine exercise intensities based on blood lactate concentrations.

Duplicate sample readings from the Lactate Plus lactate meter were strongly related, however there was a small fixed bias, indicating that the values from the second sample were consistently lower than values from the first sample. In addition, there was a very small proportional bias. Both of these biases may be explained by using separate samples collected directly from the finger. The milking of the finger to obtain a blood sample can cause the dilution of the blood sample by interstitial fluid. The manufacturer warns the user against vigorous squeezing of the finger to obtain a blood drop. The use of a vasodilating cream may resolve this issue. When we used the same mixed blood sample as the reference analyzer, the proportional bias was eliminated, while the fixed bias was reduced by approximately 65%.

We also found that the hand-held analyzer was unable to analyze the blood sample 11% of the time, presumably from an insufficient sample volume. This was surprising given that the Lactate Plus lactate meter provides an audible signal to indicate when the test strip has a sufficient volume of blood for analysis. Our experience has shown that anticipating the filling of the test strip can result in both the audible signal and an error. However, even when great care is taken, one can still get an audible full signal and the error message.

Ridenour et al. advocated for a switch from fetal blood sampling to lactate analysis.[1] However, their data showed that the variability in blood [lactate] accounted for only 46% of the variability in pH. This could be due to the significant proportional bias that is apparent in their data (Ref 1, Figures 1 and 3). However, our analysis shows a fixed and proportional bias that are less than half reported by previous studies relying on Bland-Altman plots and simple comparison of means.[1 4] This suggests the modest correlation between fetal [lactate] and blood pH is best attributed to the independent regulation of blood lactate and pH rather than unreliable measurement of [lactate].[17 18]

We did not compare the Lactate Plus lactate meter to known standards. This limits the precision with which we can quantify the accuracy of the hand-held analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. Our analysis assumes measurement error in both the hand-held and reference instrument. Thus it is likely that by comparing the Lactate Plus lactate meter directly to known lactate standards, our fixed bias would be reduced.

In summary, the Lactate Plus lactate meter is a valid and reliable instrument across a wide range of blood lactate concentrations. Any proportional or fixed bias in blood lactate concentration is nearly indistinguishable from zero. Therefore, the hand-held analyzer can be used to determine exercise intensities based on absolute or relative blood lactate concentrations. Sampling procedures can have a significant effect on the reliability of the hand-held analyzer, and the hand-held analyzer is prone to

technical issues in nearly one out of ten samples.

CONTRIBUTORS

Sarah Drevets collected and analyzed blood samples, and helped with data reduction.

Kathryn Drevets collected and analyzed blood samples, and helped with data reduction.

Micah Alford collected some data and performed statistical analysis.

Amanda Salacinski helped design the study, collected data, and revised the manuscript.

Brian E. Hunt designed the study, collected data, designed statistical analysis, and wrote the manuscript.

COMPETING INTERESTS

None

ETHICS APPROVAL

Ethics approval was provided by the Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study.

DATA SHARING STATEMEMNT

No additional data are available

ACKNOWLDGMENTS

Lactate test strips and quality assurance solutions for the Lactate Plus lactate meter were provided by Lactate.com.

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FIGURE LEGENDS

Figure 1. Determination of the Lactate threshold by visual inspection. Shown are data from a representative study participant and the lines of best fit that were determined independently for data from the YSI 2300 lactate analyzer and the Lactate Plus lactate meter.

Figure 2. Bland-Altman plot depicting the level of agreement between lactate concentrations determined by Lactate Plus hand-held analyzer the YSI bench top analyzer.

Figure 3. Ordinary least products regression analysis of the relation between lactate concentrations determined by the Lactate Plus hand-held analyzer and the YSI bench top analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equations and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 4. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the YSI bench top analyzer. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 5. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the Lactate Plus hand-held lactate analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 1

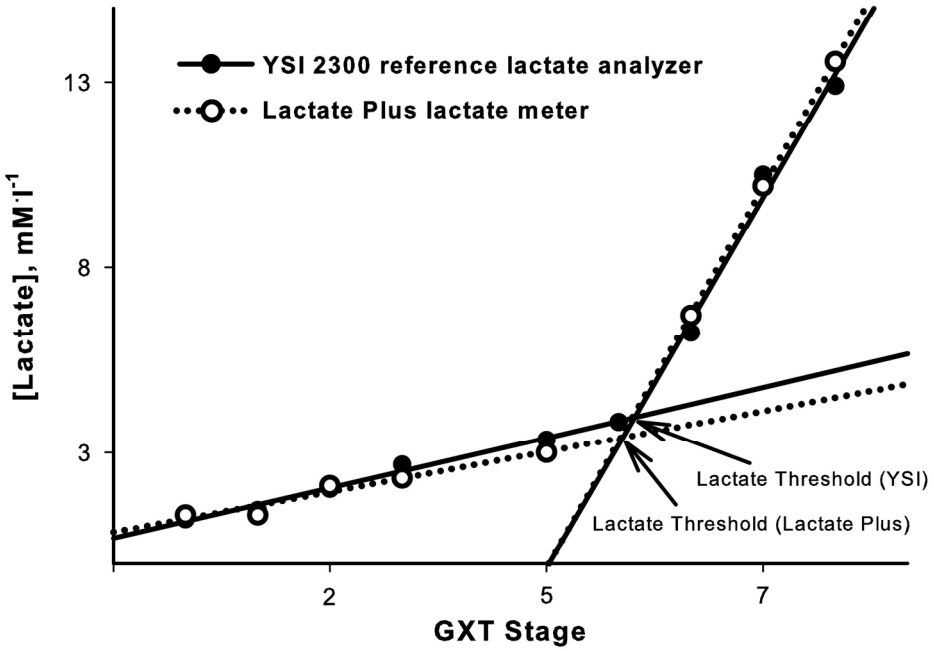

Figure 1. Determination of the Lactate threshold by visual inspection. Shown are data from a representative study participant and the lines of best fit that were determined independently for data from the YSI 2300 lactate analyzer and the Lactate Plus lactate meter.
168x178mm (300 x 300 DPI)

Figure 2

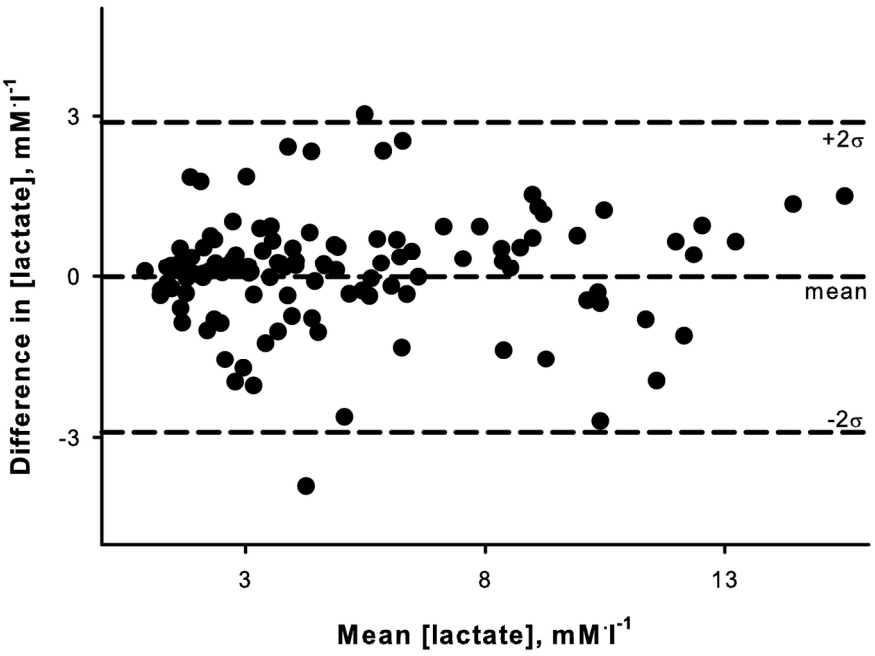

Figure 2. Bland-Altman plot depicting the level of agreement between lactate concentrations determined by Lactate Plus hand-held analyzer the YSI bench top analyzer.
179x194mm (300 x 300 DPI)

Figure 3

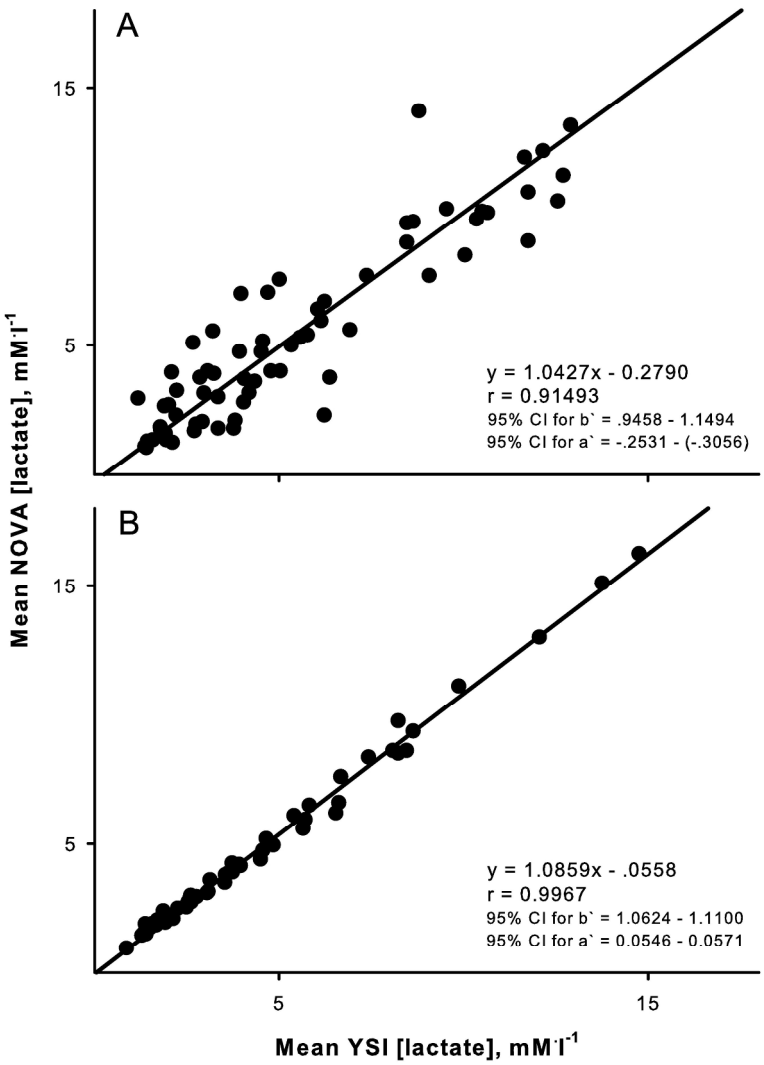

Figure 3. Ordinary least products regression analysis of the relation between lactate concentrations determined by the Lactate Plus hand-held analyzer and the YSI bench top analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equations and confidence intervals for slope (b) and y-intercept (a) are presented.
229x317mm (300 x 300 DPI)

Figure 4

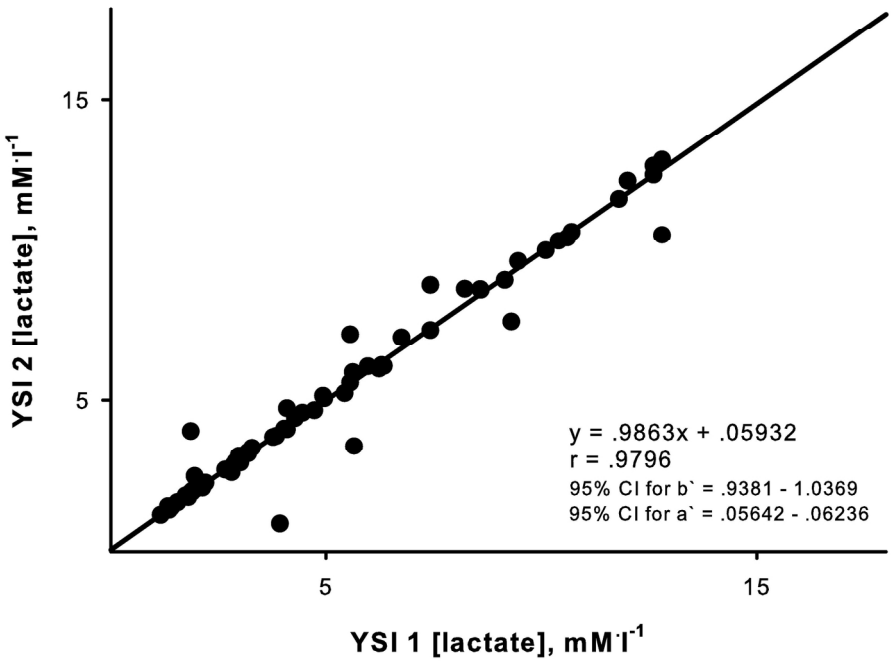

Figure 4. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the YSI bench top analyzer. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.
172x181mm (300 x 300 DPI)

Figure 5

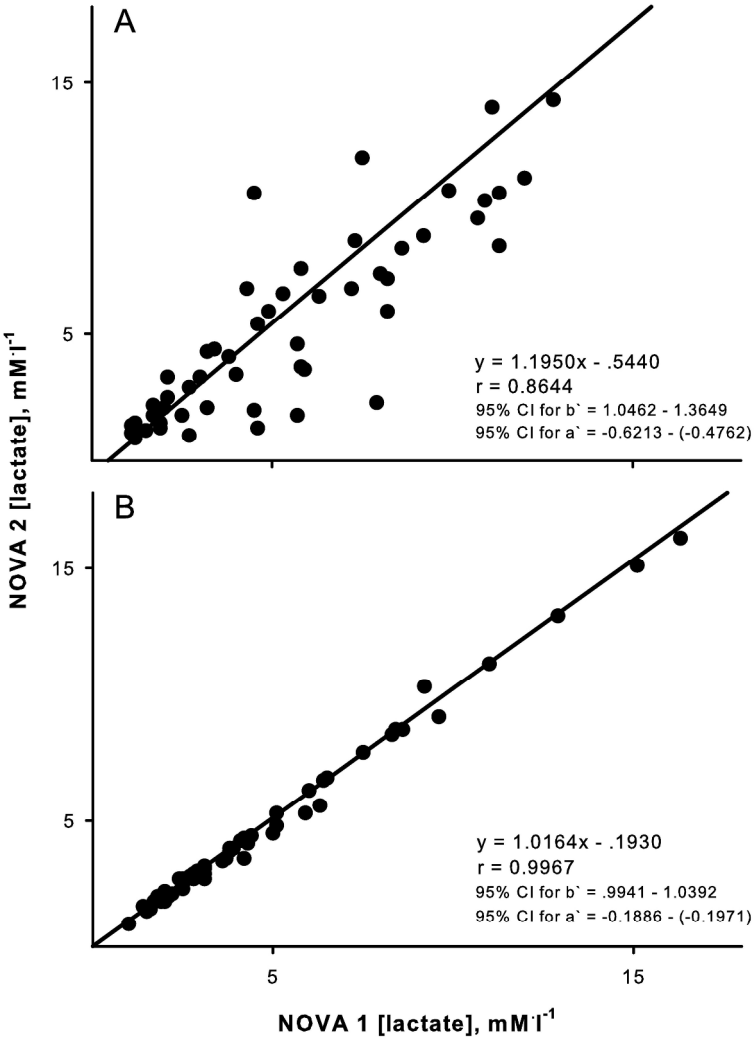

Figure 5. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the Lactate Plus hand-held lactate analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.
234x318mm (300 x 300 DPI)

The authors would like to thank Dr.'s Fernandes and Buzzachera for their insights and suggestions regarding our manuscript. We have carefully considered each comment and it's potential impact on our manuscript. We have responded to each comment below, providing the details of our changes to the manuscript, where each change can be found within the manuscript, or our rationale as to why we have not revised the manuscript as suggested by the reviewer. We believe that the reviewer's comments have helped us write an improved manuscript.

Responses to Dr. Fernandes

1. **Title could be briefer.**
   We appreciate Dr. Fernandes' sentiments, and would like to have a more concise title as well. However, we have not been able to devise a title of less than 17 words that is both adequately descriptive of the study and meets the journal's requirement to include the study design in the title. We hope that Dr. Fernades will note that our title falls well short of the 50-word limit set by the publishers of BMJOpen.

2. **"The Lactate Plus portable lactate meter provides valid and reliable measurements of blood lactate concentration". Is this new? Tanner et al (2010) did not report it before?**
   Tanner did conclude that the lactate Plus meter "displayed good reliability and accuracy…" However, Tanner's conclusions rely on a questionable analytic approach. Moreover, there clearly appears to be systematic measurement error that was not examined. If one looks at their Figure 4 (shown below) it appears as though a proportional bias exists. This is more evident in Tanner's figure 5 (also shown below). Our approach does not suffer from the assumptions inherent in Tanners analytical approach. Our use of least-products regression allows the reader to assess the accuracy and reliability based on three independent parameters: 1) correlation coefficient; 2) the degree of proportional bias; and 3) the degree of fixed bias. While we come to the same conclusion as Tanner et al, our conclusions are based on a firm analytical approach. Moreover, our approach indicates the meter is 93% more accurate than reported by Tanner et al.

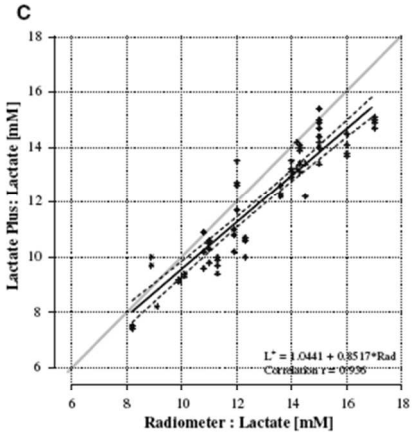

Fig. 4 Portable analyser correlation plots for Lactate Pro (**a**, *circles*), Lactate Scout (**b**, *triangles*) and Lactate Plus (**c**, *asterisks*) analysers versus Radiometer ABL 700 analyser. Linear regression is represented by *solid black line*, ±95% CI by *dashed lines* and line of identity by *grey solid line*. Linear regression equation and correlation coefficients are presented on *bottom right of graph*

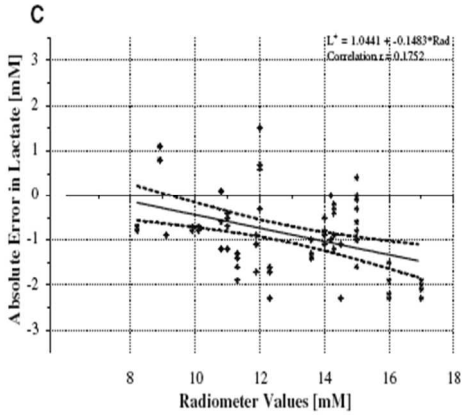

Fig. 5 Portable analyser Bland–Altman plots for Lactate Pro (**a**, *circles*), Lactate Scout (**b**, *triangles*) and Lactate Plus (**c**, *asterisks*) analysers versus Radiometer ABL 700 analyser. Linear regression is represented by *solid black line* and ±95% CI by *dashed lines*. Linear regression equation and correlation coefficients are presented on *top right of graph*

We discuss these points in the Discussion on page 10 as follows: *Though not specifically assessed, it does appear that Tanner's reported difference between the hand held and reference analyzer is significantly influenced by a proportional bias (Ref 8, Figures 4 and 5). The fact that our data shows little proportional bias (Figure 3) may account for the greater agreement between analyzers that we observed. It is possible that if Tanner had been able to independently determine the proportional and fixed bias, their analysis may have revealed a small bias similar to ours. Differences in reference instruments would not likely explain the greater measurement error reported by Tanner, given that their instrument undergoes a 3-point calibration and 2-point calibration check every few hours, similar to our reference instrument.*

Moreover, given that Tanner's figures showed a strong proportional bias, as does most other validity data from various hand-held analyzers, our study took the next obvious step, and tested whether this proportional bias was enough to affect the detection of the lactate threshold.

3. **"Objectives: The aims of this study were to: 1) determine the validity and reliability of the Nova Biomedical Lactate Plus hand-held lactate meter". My previous comment also applies here.**
   See response for comment #2 above.

4. **As the number of words was not exceeded, some details should be given: (i) which type of exercise was implemented; (ii) was the test continuous or intermittent? (iii) which methodology was used for assessing lactate threshold?**
   Your point is well taken. We have added this information to the abstract at follows: ***Design and Participants:*** *In this method comparison study 15 active men and women performed a discontinuous graded exercise test to volitional exhaustion on a motorized treadmill. …* ***Primary Outcome Measurements:*** *… Lactate threshold was determined by visual inspection.*

5. **The values of blood lactate concentration should be given in mM per liter.**
   Thank you for catching this oversight. This has been corrected throughout the manuscript and figures.

6. **"The lactate Plus analyzer provides accurate and reproducible measurements… that can be used to estimate workloads corresponding to blood lactate concentration transitions or absolute lactate concentrations". And what about exercise intensities under lactate threshold? Could this analyzer also be used for light-moderate exercise prescription?**
   As is implicit in our statement quoted above, the lactate measurements from the hand-held analyzer can be used for estimating workloads at *any* absolute lactate concentration. However, we have revised the last sentence in the abstract to make this point more explicit as follows: ***Conclusion:*** *The Lactate Plus analyzer provides accurate and reproducible measurements of blood lactate concentration that can be used to estimate workloads corresponding to blood lactate transitions or any absolute lactate concentrations.*

7. **"...has also been proposed as a measure of metabolic acidosis during fetal examinations". Is this relevant for the current study? Were these examinations carried on with protable hand-held lactate meters? Authors should consider removing this example.**
We can understand Dr. Fernandes' point, but we included this point for 2 reasons: 1) to help the reader understand that blood lactate measurement is important beyond the narrow field of athletic performance; and 2) to help the reader understand why this paper is appropriate for publication in BMJ. To answer Dr. Fernandes 2nd question, the study cited (Ridenour et al.) specifically used the Lactate Plus lactate meter to measure blood lactate concentration in order to indicate fetal acidosis.

8. **Please provide the range of values for sample of blood for bench top analyzers as done for portable hand-held lactate meters.**
Thank you for helping us stay consistent in the development of our thoughts. We have revised the Introduction to as follows: *Portable hand-held lactate meters have advantages over bench top models including: 1) their ability to rapidly sample blood lactate concentration ([lactate]), in or outside the laboratory, 2) they require a much smaller sample of blood (0.5 – 0.7μl) than many bench top analyzers (25 – 75μl), and 3) they can be purchased and operated at a lower cost than many bench top models.*

9. **"...difference between the reference and hand-held analyzer can be as much as 1.0 mM" and "represent nearly 10% of the full range of values in some Populations". A reference is welcome here.**
This certainly would not be true for highly trained athletes, but can be true for sedentary or untrained individuals. We have included an appropriate reference as follows: *While the majority of studies report the [lactate] measured using hand-held analyzers is similar to those of various bench top models, the mean difference between the reference and hand-held analyzer can be as much as 1.0 mM·l^{-1}. This can represent nearly 10% of the full range of values in some populations.* [9] (Juel C, Klarskov C, Nielsen JJ, Krustrup P, Mohr M, Bangsbo J. Effect of high-intensity intermittent training on lactate and H+ release from human skeletal muscle. *Am J Physiol Endocrinol Metab* 2004;286(2):E245-51.)

10. **"This level of disagreement could be explained by the presence of systematic error, which has gone unexamined in previous studies". As Baldari et al (ref#2) has examined systematic error, authors are advised to rewrite this sentence. In addition, authors also refer two studies (refs #9 and 10) that studied this topic.**
We respectfully disagree. Perhaps the authors and Dr. Fernandes are using the term "systematic error" differently. We explicitly define our use of the term in the introduction based on the definition of Ludbrook (refs 10 and11). We also clearly describe the biases produced by systematic measurement error, which previous studies have not examined. For example, it is clear to us that the data from Baldari et al. displays a proportional bias as shown in their Figure 2, shown near the top of the next page. Moreover, in Figure 3 Baldari reports regression slopes between 0.938 and 1.105, yet does not report whether these slope are significantly different from 1.0 (indicative of a proportional bias). Thus, Baldari did not look for evidence of systematic measurement error in their data. Furthermore, references 9 and 10 (now refs 10 and 11), to which Dr. Fernandes refers do not examine systematic

measurement error in hand-held lactate meters, but are papers describing the advantages of least products regression over the least squares regression approach used by Baldari and most other authors that have performed validation studies on these hand-held meters.

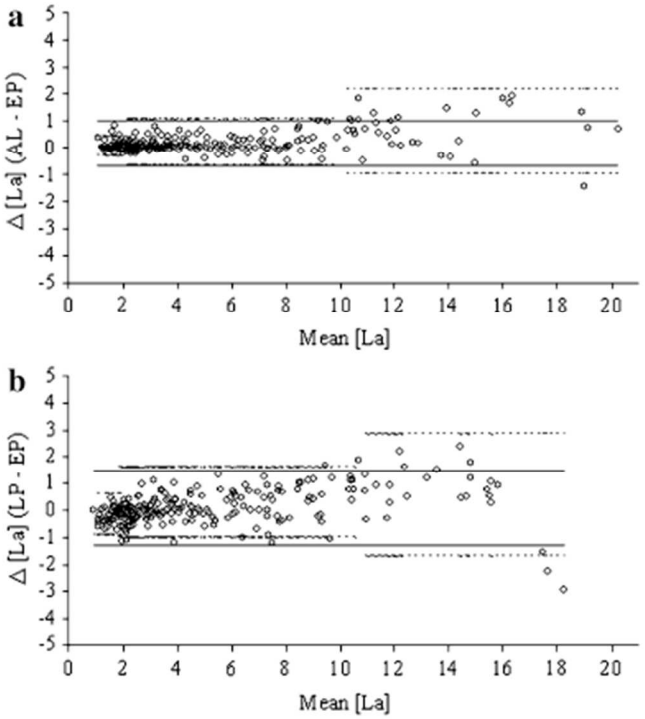

Fig. 2 Bland-Altman plots showing limits of agreement between blood lactate concentrations expressed in mM ([La]) measured using the EBIO plus, Accutrend and Lactate Pro analyzers. *Dashed lines* are the limits of agreement for low, medium, high [La]. **a** Relationship of mean [La] determined by Accutrend and EBIO plus with the difference in La between analyzers (Δ [La] (AL-EP)). **b** Relationship of mean [La] determined by Lactate Pro and EBIO plus with the difference in La between analyzers (Δ [La] (LP-EP))

11. "Hand-held meters..., are designed to sample blood directly from a finger". This idea is repeated through the manuscript. However, blood collection from the ear lobe is also very common. Please re-phrase.

We understand the reviewer's perspective. Therefore, we have revised this statement on page 5 to read as follows: *Hand-held meters, however, are designed to sample blood directly from a puncture for ease of use in the field.*

12. "using a finger stick to draw blood it is not uncommon to require "milking" of the finger to get an adequate sample". Was this described before or is from authors' personal experience? This problem can be solved using a vasodilator cream.

The effects of milking of the finger to produce a blood drop for sampling has been most extensively studied with hand-held glucose meters. For example see Fruhstorfer and Quarder. Diabetes Res Clin Pract 85(1), e14-15, 2009. Moreover, the manufacturer of the Lactate Plus meter specifically advises users that if they must squeeze the finger to form a drop of blood "do not squeeze vigorously." We acknowledge that a vasodilating cream could be used to minimize or eliminate the need for milking of the finger, though this is extremely rare in the literature. We have revised the Discussion on page 11 to read: *The milking of the finger to obtain a blood sample can cause the dilution of the blood sample by interstitial fluid. The manufacturer warns the user against vigorous squeezing of the finger to obtain a blood drop. The use of a vasodilating cream may resolve this issue.*

13. "Given that duplicate samples are standard practice". Was this described before or is from authors' personal experience?

This is a "best practice" based on statistical principles as well as the relatively large differences reported by investigators such as Baldari (SEE = 0.55 mM.l-1) and Tanner (0.9 mM.l-1). Nonetheless, we have removed the statement from the Introduction on page 5 and the Discussion on page 11.

14. After the specific aims of the study, some hypotheses are welcome.

Typically, validity and reliability studies are not hypothesis driven (see Ref. 1 – 8).

15. Lactate analysers are, as referred by the authors, a very important instrument to help in training control and prescription of endurance athletes. Nevertheless, the subjects used in the current study do not seem representative of the high trained athletes. This fact could lower the overall quality of the paper.

We disagree that the training status of the study participants has any relevance to this paper. The aim of this study was to assess the accuracy and reliability of the Lactate Plus analyzer. It is unclear why the device would accurately measure lactate concentration in one population and not in another.

16. Units should be abbreviated as proposed in SI Units (eg min and s).

Again, thank you for bringing this oversight to our attention. These abbreviations, where they occur, now conform to those proposed for SI units.

17. The portable lacate meter used in the current study was designated in three different forms along the text: Nova Biomedical Lactate Plus, Lactate Plus and Lactate Plus (Nova Biomedical). Please be consistant.

We agree that this can be distracting to the reader. We now consistently refer to the lactate meter as Lactate Plus lactate meter.

18. "As per the manufacturer instructions we used a low…" Please rewrite.

We have revised the sentence on page 6 to read as follows: *Following the manufacturer instructions we used a low (1.0 – 1.6 mM·l^{-1}) and high (4.0-5.4 mM·l^{-1}) quality control solution to ensure the lactate meter was operating properly at the beginning of each data collection session.*

19. "For the first…YSI 2300". This section is hard to follow. Please rewrite.

This was indeed a difficult section to write. We appreciate another opportunity to make our writing more clear. We have revised this section on page 6 to read as follows: *For the first nine participants three blood samples were taken directly from the finger between each stage of the graded exercise test (GXT). All samples were taken in this order: 1) hand-held directly from finger, 2) capillary tubes for the YSI 2300 from the finger, and 3) a second sample directly from finger using the hand-held meter. To assess the effect of blood sampling techniques on the accuracy of the hand-held meter blood was drawn from the finger into capillary tubes and allocated to both the YSI 2300 and hand-held meter for the last six participants.*

20. Please provide treadmill reference.

We have provided the reference on page 6 as follows: *Participants performed a discontinuous graded exercise test (GXT) on a motorized treadmill (Quinton TM65).*

21. I wonder why it was used a discontinuous graded exercise test since the continuous one is the most proper for assessing physiological parameters (e.g. oxygen consumption, heart rate and blood lactate concentrations) and it is not necessary to stop the exercise to collect blood when performing on a treadmill. Please justify your choice.

We agree that blood samples can be collected while the subject is walking or running on the treadmill. However, we chose to use a discontinuous protocol because we were not collecting a single blood sample, but three samples. Thus, in our pilot testing we found that a discontinuous protocol allowed us to collect all three samples during the 1-minute sampling period.

22. Please explain it were not used fixed protocol increments. Was this protocol previously described in the literature?

This GXT protocol has not been previously described in the literature. It is unclear to the authors how our protocol would negatively affect our ability to assess the accuracy and reliability of the hand-held analyzer, or model changes in blood lactate concentration.

23. "...Bland-Altman plots were constructed to allow the reader to ..." Authors choose their statistical procedures based on scientific principles or in the readers opinion? Please rewrite.

We agree that statistical analyses should be chosen based on the experimental question or hypothesis being tested and statistical principles. However, we also believe that an important aspect of writing a scientific paper is to inform the readers. This includes helping the readers understand our findings within the context of previous work. Therefore, we chose to construct a Bland-Altman plot because this has become commonplace in methodological studies. (see ref 1-6,8) So as a service to our readers, we provide a common point of comparison between our data and those previously published.

24. The 1st paragraph of the Data Analysis section is too descriptive. In our opinion, it should be briefer and some references should be added.

Based on the analytic approaches used in previous validation studies, it is reasonable to assume that a thorough explanation of our approach is warranted. We have added references to this section on page 7 as follows: *Validity was determined from the correlation coefficient in combination with the presence and degree of bias. The degree of fixed bias was determined from the y-intercept 95% confidence intervals. If the confidence interval for the intercept includes the value of zero, then there is no fixed bias. Proportional bias was determined from the 95% confidence interval for the slope. If the confidence interval for the slope includes the value of 1.0, then there is no proportional bias. Ordinary least products regression gives different slopes and y-intercepts than does least squares regression because error is assumed in both hand-held and bench top analyzers.* [10 11]

25. As it is well described that after lactate threshold intensity of blood lactate concentrations assumes an exponential increase, we wonder if the use of 2 linear regressions in the best way to assess lactate threshold. If authors want to go deep in this topic, the can consult a study of our group (Fernandes RJ et al. Individual Anaerobic Threshold in Swimming, Int J Sports Med 2011; 32: 940-946).

This is the one common concern shared by the reviewers, and we agree this is an issue that needs to be addressed. We chose to follow the procedures outlined by Gaskill et al (Med Sci Sports Exerc 2001; 33(11):1841-48) as suggested by Dr. Buzzachera. This has slightly reduced the correlation coefficient and changed the parameters of the regression line. We have clarified our approach in the Methods section on page 7 as follows: *Lactate threshold was defined as the point at which blood [lactate] began to increase in a non-linear fashion.*[12 13] *The threshold was estimated by plotting [lactate] against GXT stage. These graphs were visually inspected to determine the lines of best fit by the two evaluators. The equations for each line were set equal to one another and solved for the point of intersection (Figure 1). The values from each evaluator were averaged.* [14]

We have also revised our results accordingly on page 8 as follows: *there was excellent agreement between estimates of the lactate threshold based on lactate values from the hand-held lactate meter compared to those from the bench top analyzer (r = 0.97). Moreover, there was neither a proportional bias (95% CI for slope: 0.910 to 1.098), nor a fixed bias (95% CI for y-intercept: -0.396 to 0.325) in estimates of the lactate threshold from the hand-held analyzer.*

26. The use of fixed blood lactate concentrations of 2.5 and 4.0 mM/l should be justified. Why not 3.5 mM/l, as proposed by Heck et al (Int J Sports Med 1985; 6: 117-130) for lactate threshold, or 8.0 mM/l that is considered a good indicator of aerobic power?

The reviewer's point is well taken. Many investigators use several different absolute lactate values to quantify blood lactate concentration. We have added references to support our use of 2.5 and 4.0 $mM \cdot l^{-1}$ on page xx as follows: *These equations were also used to calculate the stage that corresponded to an absolute blood [lactate] of 2.5 and 4.0 $mM \cdot l^{-1}$.*[14 15]

27. Fig 1: if this is an example of a subject please clearly state it. Moreover, if is important to check if the number of points for the YSE and Lactate Plus are correct (6 and 8, respectively).

We have revised the figure legend on page 16 to indicate these data are from a study participant as follows: *Figure 1. Determination of the Lactate threshold by visual inspection. Shown are data from a representative study participant and the lines of best fit that were determined independently for data from the YSI 2300 lactate analyzer and the Lactate Plus lactate meter.*

We were not able to collect any blood after stage 4 and could not get a blood sample with the hand-held analyzer after stage 7. Thus, the YSI data set contains 9 data points and the Lactate Plus data set contains 8 points. Values for rest and stages 1 and 2 are nearly identical and are difficult to distinguish.

28. It is stated that from the 242 blood samples taken using the hand-held analyzer, 27 resulted in error messages due to insufficient sample. This is odd once some portable analyzers emit an auditory signal when the quantity of blood is sufficient. Comment Please.

We agree it is odd that the auditory signal can sound and yet still give an error message that is associated with inadequate sample volume. This may be due to operator error, though even when care is taken this still occurs. We have revised the Discussion on page 11 to expand on this point as follows: *We also found that the hand-held analyzer was unable to analyze the blood sample 11% of the time, presumably from an insufficient sample volume. This was surprising given that the Lacate Plus meter provides an audible signal to indicate when the test strip has a sufficient volume of blood for analysis. Our experience has shown that anticipating the filling of the test strip can result in both the audible signal and an error. However, even when great care is taken, one can still get an audible full signal and the error message.*

29. "However, differences of almost 1.0mM can significantly impact the use of absolute [lactate] to characterize training intensity or efficacy". This topic should be better developed since it is important to evidence why differences of ~1.0mM/l are so important for training characterization.

We appreciate the point made by Dr. Fernandes. We have developed our point more fully in the Discussion on page 10 as follows: *However, differences of almost 1.0 $mM \cdot l^{-1}$ can significantly impact the use of absolute [lactate] to characterize training intensity or efficacy. Weltman et al. reported that women who trained at an intensity corresponding to about 2.5 $mM \cdot l^{-1}$ showed greater improvement in blood lactate parameters, but less of an improvement in VO2max than did women training at their lactate threshold.* [15] *If true,*

then an error in the measurement of blood lactate concentration could lead to suboptimal improvements in either lactate parameters or VO2max.

30. **Although not being the main focus of the current research, it seems important to give the mean (SD) values for blood lactate concentrations corresponding to lactate threshold. As referred in the text, this parameter is of fundamental importance for endurance athletes; so, it should be presented (and discussed).**
The purpose of estimating the lactate threshold was to determine if the proportional bias we anticipated seeing was large enough to affect the estimation of the lactate threshold or other lactate parameters found in the literature. Thus, it seems to us that the mean value and variability of lactate thresholds within our study sample irrelevant to the aims of our study and interpretation of our data. If we were trying to draw some conclusion about the "eliteness" of our study sample it would certainly make sense, but that is not the case here.

31. **Please consider to include some relevant studies in accordance with the previous comments.**
As can be seen from our responses above, several references have been added to address Dr. Fernandes' concerns.

Responses to Dr. Buzzachera

1. **Page5, Lines 34: It should be noted the study sample must be enough to validate any instrument. So what about sample size? I believe a sample size calculation should be included in the Methods section.**
Dr. Buzzachera's point is correct; a sample size calculation should have been done a priori. However, a post-hoc sample size calculation is inappropriate. The concern now would be if we reported clinically significant differences, say close to 1 mM.l-1 and reported that our analysis indicated this was not statistically different from zero. This would be indicative of a sample size problem. As can be seen by the results, our sample size was adequate to see a difference of 0.056 $mM \cdot l^{-1}$, a difference that is 93% smaller than had previously been reported. Thus, our sample size seems more than adequate given our statistical approach.

2. **Page7, Lines 43: There is concern with the procedures used to identify the lactate threshold of the participants. In particular, the authors have stated "the threshold was estimated by plotting [lactate] against GXT state. These graphs were visually inspected to determine the lines of best fit". However, other procedures should be conducted to correctly identify lactate threshold. For example, the visual interpretation of each graph should be independently (and preferentially) made by at least two trained researchers to locate "the point at which blood [lactate] began to increase in a nonlinear fashion" (Beaver' method, J Appl Physiol, 1985). If the independent determinations of the state at lactate threshold differ between researchers, a third researcher should adjudicate the difference by independently determining lactate threshold. The three researchers then jointly should agree on the lactate threshold point. If no agreement about the lactate threshold point occurs, data should be rejected (Gaskill at al., Med Sci Sports Exerc, 2001). The authors are encouraged to clearly explain how the visual inspection of the graphs to**

identification of the lactate threshold was carried out in the investigation under review. If no procedures as previously cited were conducted, I believe that, at a minimum, this problem should be acknowledged as a limitation to this study.

Thank you for your comments and guidance. This is the one common concern between reviewers. Please see our response to Dr. Fernandes' comment # 25.

3. **Strength and Limitations Section: The sentence "We did not compare either instrument to known lactate standards. This may limit our ability to precisely quantify the accuracy of the hand-held analyzer…" should be included within the Discussion section. I believe this suggestion could be useful for allowing a better comprehension of this limitation by reviewers and future readers.**

We understand Dr. Buzzachera's suggestion that this limitation also appear in the discussion, as it should. Thus, we have added this limitation and a further explanation to the Discussion section on page 12 as follows: *We did not compare the Lactate Plus lactate meter to known standards. This limits the precision with which we can quantify the accuracy of the hand-held analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. Our analysis assumes measurement error in both the hand-held and reference instrument. Thus it is likely that by comparing the Lactate Plus lactate meter directly to known lactate standards, our fixed bias would be reduced.*

A method-comparison study regarding the validity and reliability of the Lactate Plus analyzer

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A method-comparison study regarding the validity and reliability of the Lactate Plus analyzer

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SUMMARY

Article focus

- Determine the validity and reliability of the Lactate Plus analyzer and quantify any systematic bias.
- Determine the effect of any bias on the determination of lactate threshold
- Determine the effect that blood sampling methods have on validity and reliability

Key messages

- The Lactate Plus analyzer provides valid and reliable measurements of blood lactate concentration.
- The Lactate Plus analyzer demonstrates a small fixed and proportional bias.
- Sampling directly from the finger does increase the variability in measurement, likely due to the milking of the finger rather than the analyzer itself.

Strengths and limitations

- This study compares the accuracy and variability in measurements under both laboratory and field sampling conditions.
- We used least-product regression analysis to independently quantify fixed and proportional bias rather than Bland-Altman plots or least-squares regression, which lump these bias together or assumes there is no measurement error in the reference method.
- We did not compare either instrument to known lactate standards. This may limit our ability to precisely quantify the accuracy of the portable analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. This reduces the likelihood that our reference instrument is inaccurate or non-linear.

ABSTRACT

Objectives: The aims of this study were to: 1) determine the validity and reliability of the Nova Biomedical Lactate Plus portable analyzer, and quantify any fixed or proportional bias, 2) determine the effect of any bias on the determination of the lactate threshold, and 3) determine the effect that blood sampling methods have on validity and reliability. **Design and Participants:** In this method comparison study 15 active men and women performed a discontinuous graded exercise test to volitional exhaustion on a motorized treadmill. Blood samples were taken via finger prick and collected in micro capillary tubes for analysis by the reference instrument (Yellow Springs Instrument 2300 Glucose and Lactate Analyzer) at the end of each stage. Duplicate samples for the portable analyzer were either taken directly from the finger or from the micro capillary tubes. **Primary Outcome Measurements:** Ordinary least products regressions were used to assess validity, reliability, and bias in the portable analyzer. Lactate threshold was determined by visual inspection. **Results:** Though measurements from both instruments were correlated (r=0.91), the differences between instruments had large variability (SD = 1.45 $mM \cdot l^{-1}$) when blood was sampled directly from finger. This variability was reduced by ~95% when both instruments measured blood collected in the capillary tubes. As the proportional and fixed bias between instruments was small, there was no difference in estimates of the lactate threshold between instruments. Reliability for the portable instrument was strong (r=0.99, $p<0.05$) with no proportional bias (slope=1.02) and small fixed bias (-0.19 $mM \cdot l^{-1}$). **Conclusion:** The Lactate Plus analyzer provides accurate and reproducible measurements of blood lactate concentration that can be used to estimate workloads corresponding to blood lactate transitions or any absolute lactate concentrations.

INTRODUCTION

Not only is blood lactate accumulation a common measure in the physiological assessment of endurance athletes, but is also an important clinical measure.[1-4]Portable lactate analyzers have advantages over bench top models including: 1) their ability to rapidly sample blood lactate concentration ([lactate]), in or outside the laboratory, 2) they require a much smaller sample of blood (0.5 – 0.7µl) than many bench top analyzers (25 – 75µl), and 3) they can be purchased and operated at a lower cost than many bench top models.

Several studies have attempted to evaluate the validity and reliability of these portable analyzers.[3-10] While the majority of studies report the [lactate] measured using portable analyzers is similar to those of various bench top models, the mean difference between the reference and portable analyzer can be as much as 1.0 $mM \cdot l^{-1}$. This can represent nearly 10% of the full range of values in some populations. [11] This level of disagreement could be explained by the presence of systematic measurement error. Systematic measurement error can result in a proportional bias, where one instrument produces values that are different from those of another instrument by an amount that is proportional to the level of the measured variable, and/or a fixed bias, where one instrument gives values that are different from those of another instrument by a constant amount.[12 13] Thus, similar mean values between lactate analyzers could occur while the portable analyzer produces low values at lower [lactate] and high values at higher [lactate] or vice-versa. Previous studies have primarily relied on Bland-Altman analysis to determine the presence of any fixed bias. However, this approach does not allow the independent determination of bias, and thus has limited utility in assessing the presence of systematic measurement error. Therefore, while most data appear to show a substantial proportional and/or fixed bias the presence and degree of bias in portable lactate analyzers remains unresolved.[3 4 6-10] Furthermore, because previous studies have not directly examined these biases it is unclear if they are large enough to

affect estimates of various lactate parameters, such as pH or lactate threshold.

Blood sampling techniques may also affect measurement accuracy and reliability. Previous studies have either used intravenous blood drawn directly into a syringe,[3 7 9] or capillary blood from a finger stick drawn into capillary tubes then mixed as would be done in the laboratory.[6 10] Portable analyzers, however, are designed to sample blood directly from a puncture for ease of use in the field. When using a finger stick to draw blood it is not uncommon to require "milking" of the finger to get an adequate sample. This may dilute the lactate concentration by increasing interstitial fluid in the sample. It would seem important to understand and quantify the effect of differing blood-sampling procedures on the accuracy and reliability of these portable analyzers.

Given the questions that remain regarding the validity and reliability of portable lactate analyzers the specific aims of the present study were: 1) to determine the validity and reliability of the Lactate Plus analyzer (Nova Biomedical), and quantify any fixed and/or proportional bias, and 2) determine the effect that blood sampling methods have on validity and reliability.

METHODS

Fifteen young (20-36 yr.; mean = 24.5 yr.) men and women (6 women) participated in the study. All subjects reported at least 90 minutes of moderate to vigorous physical activity each week. All subjects read and signed an informed consent. The Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study. All procedures conformed to the Declaration of Helsinki.

Instruments

To determine the validity of the Lactate Plus analyzer we used the YSI 2300 Stat Plus Glucose

and Lactate analyzer from Yellow Springs Instruments (Yellow Springs, OH) as our reference instrument. This bench top laboratory analyzer uses a membrane-bound enzyme electrochemical methodology. L-lactate oxidase is immobilized in a thin membrane placed over an electrochemical probe. The enzyme catalyzes the conversion of L-lactate to pyruvate and hydrogen peroxide, the latter then being oxidized at the platinum anode to measure lactate concentration in whole blood or plasma. A new membrane was used for each data collection session. The analyzer was initially calibrated using a 5 $mM \cdot l^{-1}$, 15 $mM \cdot l^{-1}$, and 30 $mM \cdot l^{-1}$ solution. In addition, an automated quality control was performed in triplicate every 45 minutes using a 5 $mM \cdot l^{-1}$ solution. Blood samples were collected from a finger stick into two heparinized capillary tubes. Blood was then mixed in a micro centrifuge tube. Two 25µl samples were sequentially aspirated and measured by the analyzer.

The Lactate Plus analyzer uses an electrochemical lactate oxidase biosensor to measure lactate concentration in a 0.7 µl sample. Following the manufacturer instructions we used a low (1.0 – 1.6 $mM \cdot l^{-1}$) and high (4.0-5.4 $mM \cdot l^{-1}$) quality control solution to ensure the lactate analyzer was operating properly at the beginning of each data collection session. For the first nine participants three blood samples were taken directly from the finger between each stage of the graded exercise test (GXT). All samples were taken in this order: 1) portable directly from finger, 2) capillary tubes for the YSI 2300 from the finger, and 3) a second sample directly from finger using the portable analyzer. To assess the effect of blood sampling techniques on the accuracy of the portable analyzer blood was drawn from the finger into capillary tubes and allocated to both the YSI 2300 and portable analyzer for the last six participants.

Graded Exercise

Participants performed a discontinuous graded exercise test (GXT) on a motorized treadmill

(Quinton TM65). Each stage lasted two-minutes with a one-minute blood sampling period between stages. The finger was prepared for sampling just prior to the end of each exercise stage. During the 1-minute blood collection period participants straddled the treadmill belt while blood samples were taken from a finger. After one minute the participants resumed exercise at a higher speed or grade. The initial speed was 1.55 $m \cdot s^{-1}$ and 0% grade. The speed was increased either 0.50 or 0.67 $m \cdot s^{-1}$ for each stage until the participant's heart rate was at least 80% of their age-predicted maximum (220-age). After this point the speed remained constant while grade was increased 2.5% for each stage. Exercise continued until volitional exhaustion.

Data Analysis

Two methods were used to assess validity. First, a Bland-Altman plot was constructed to allow the reader to more directly compare our data to that of previous studies since this is the approach typically used. However, because fixed and proportional biases cannot be determined independently from these plots, ordinary least products regression analysis was used. Validity was determined from the correlation coefficient in combination with the presence and degree of bias. The degree of fixed bias was determined from the y-intercept 95% confidence intervals. If the confidence interval for the intercept includes the value of zero, then there is no fixed bias. Proportional bias was determined from the 95% confidence interval for the slope. If the confidence interval for the slope includes the value of 1.0, then there is no proportional bias. Ordinary least products regression gives different slopes and y-intercepts than does least squares regression because error is assumed in both portable and bench top analyzers. [12] [13]

Lactate threshold (LT) was defined as the point at which blood [lactate] began to increase in a non-linear fashion.[14] [15] The threshold was estimated by plotting [lactate] against GXT stage. These

graphs were visually inspected to determine the lines of best fit by the two evaluators. The following guidelines were used to help guide the evaluators: 1) at least 3 data points were included in each line, 2) both lines contained unique data points, and 3) lines were chosen that produced the highest R^2 with the smallest confidence intervals. Once the lines were chosen the equations for each line were set equal to one another and solved for the point of intersection (Figure 1). The values from each evaluator were averaged.[16] These equations were also used to calculate the stage that corresponded to an absolute blood [lactate] of 2.5 and 4.0 $mM \cdot l^{-1}$. A t-test for paired data was used to compare means between analyzers. A p-value of <0.05 was considered statistically significant.

Reliability was determined using ordinary least products regression to quantify the relationship between sequential measurements for both instruments.

RESULTS

Validity

Lactate values during graded exercise ranged from 1.2 to 16.4 $mM \cdot l^{-1}$. When both portable and bench top blood samples are each taken directly from the finger the mean difference between [lactate] measured by the portable analyzer and the bench top analyzer was small across the full range of lactate values as depicted in Figure 2. While the mean difference between the two instruments was near zero, differences between the instruments had a large variability (SD = 1.45 $mM \cdot l^{-1}$). Even though there can be large differences between values measured by the portable and bench top analyzer, the paired measurements were highly correlated as shown in Figure 3A. Least products regression indicated a small fixed bias (y-intercept = -0.28 $mM \cdot l^{-1}$) between [lactate] measured with the portable and bench top analyzers. There was no evidence of a proportional bias (95% CI = 0.94 to 1.15). When the same mixed blood sample was used by both analyzers, the fixed bias was reduced to -0.056 $mM \cdot l^{-1}$, while a small

proportional bias was evident (slope = 1.08) as shown in Figure 3B.

Regardless of blood sampling approach there was excellent agreement between estimates of the LT based on lactate values from the portable analyzer compared to those from the bench top analyzer (r = 0.97). Moreover, there was neither a proportional bias (95% CI for slope: 0.910 to 1.098), nor a fixed bias (95% CI for y-intercept: -0.396 to 0.325) in estimates of the lactate threshold from the portable analyzer. Given the lack of bias it is not surprising there was no difference between blood [La] at the LT (2.88_{NOVA}±0.53 vs. 3.15_{YSI}±0.46 $mM \cdot l^{-1}$; p=0.32). In addition the stages corresponding to absolute blood lactate values of 2.5 $mM \cdot l^{-1}$ (2.99_{NOVA} vs. 2.92_{YSI}) and 4.0 $mM \cdot l^{-1}$ (4.64_{NOVA} vs. 4.61_{YSI}) were not different between portable and bench top values (p = 0.86 for both).

Reliability

The relationship between duplicate measurements of [lactate] by the bench top analyzer was very strong (r=0.99, p<0.05). Ordinary least products regression indicated no proportional bias (slope = 0.99), and a small fixed bias (0.059 $mM \cdot l^{-1}$; Figure 4). Ordinary least products regression revealed a small proportional (slope = 1.20) and fixed bias (-0.54 $mM \cdot l^{-1}$; Figure 5A) when the two duplicate blood samples for the portable analyzer were taken directly from the finger. Thus, the reading from the second sample was typically lower than from the first. However, when two duplicate measurements were taken from the same mixed blood sample, their was no proportional bias (slope = 1.02) and the fixed bias was reduced to -0.19 $mM \cdot l^{-1}$).

A total of 242 blood samples were taken using the portable analyzer. Twenty-seven of these attempts resulted in error messages (E-4 – insufficient sample). Thus, about 1-in-10 measurement attempts resulted in errors.

DISCUSSION

There were three new findings in our study: 1) The very small proportional bias indicates that the Lactate Plus analyzer is a highly linear instrument, 2) multiple blood samples directly from the finger increases measurement error, and 3) the small proportional and fixed bias in the portable analyzer does not affect the ability to determine the lactate threshold.

We chose to use ordinary least products regression to characterize the relation between the Lactate Plus analyzer and our reference analyzer. Most studies have employed a combination of Bland-Altman plots and least squares regression to determine the degree of agreement between various portable analyzers and a corresponding reference analyzer.[3-10] The mean difference between analyzers, as determined through Bland-Altman plots, is determined by the interaction of any fixed and proportional bias. Therefore, the mean difference between methods does not solely reflect the accuracy or fixed bias of the device, but in some cases, the presence of a proportional bias or loss of linearity. The use of least squares regression to characterize the level of proportional bias, as reflected in the slope of the linear relation, is skewed because all error is assigned to the dependent variable, in this case the portable analyzer. The use of least products regression to compare methods avoids both of these issues, allowing independent and more accurate determination of any fixed or proportional bias.[12 13 17]

Numerous studies have compared blood lactate measured with various portable analyzers to several different bench top analyzers.[4 5 7-10] All have reported that these portable analyzers produce similar lactate values compared with their bench top counterparts with average differences ranging from -0.8 to 1.0 $mM \cdot l^{-1}$. However, differences of almost 1.0 $mM \cdot l^{-1}$ can significantly impact the use of absolute [lactate] to characterize training intensity or efficacy. Weltman et al. reported that women who trained at an intensity corresponding to about 2.5 $mM \cdot l^{-1}$ showed greater improvement in blood lactate

parameters, but less of an improvement in VO2max than did women training at their lactate threshold. [18] If true, then an error in the measurement of blood lactate concentration could lead to suboptimal improvements in either lactate parameters or VO2max. Of the two studies that have tested the Lactate Plus analyzer, only Tanner et al. [10] reported the absolute difference between this portable analyzer and a reference analyzer (-0.8 $mM \cdot l^{-1}$). Our data show a much smaller difference between the Lactate Plus analyzer and the YSI bench top analyzer (fixed bias = -0.056 $mM \cdot l^{-1}$). Though not specifically assessed, it does appear that Tanner's reported difference between the hand held and reference analyzer is significantly influenced by a proportional bias (Ref 8, Figures 4 and 5). The fact that our data shows little proportional bias (Figure 3) may account for the greater agreement between analyzers that we observed. It is possible that if Tanner had been able to independently determine the proportional and fixed bias, their analysis may have revealed a small bias similar to ours. Differences in reference instruments would not likely explain the greater measurement error reported by Tanner, given that their instrument undergoes a 3-point calibration and 2-point calibration check every few hours, similar to our reference instrument.

Given that we found a very small proportional bias the estimation of the LT from [lactate] measured by the Lactate Plus analyzer agreed very well with those determined from [lactate] measured by the reference analyzer. Moreover, given the small fixed bias, it was not surprising that the lactate values from the portable analyzer provided similar estimates of the workload corresponding to the 2.5 $mM \cdot l^{-1}$ and the 4.0 $mM \cdot l^{-1}$ absolute lactate concentrations. These lactate concentrations were chosen because they have both sport and clinical significance. [1 2 19 20] The strong correlation coefficient and small biases suggest that the Lactate Plus analyzer can be used to accurately determine exercise intensities based on any blood lactate parameter.

Determination of the LT by visual inspection has come under scrutiny. [21 22] To reduce

subjectivity our approach to visual inspection is guided by several principles similar to those used by others. [16 23] Several methods of assessing the LT have been proposed that purport to be more objective. [14 16 24] However, many of these methods are known to be significantly affected by data outliers and/or missing data. [25 26] Therefore, the choice of any analytical approach has a subjective component. While our approach likely produces LT values that are different from other approaches, it produced values consistent with other studies that employed similar approaches to LT estimation. [18 23] When one considers the strong correlation and small biases in our data, it seems likely the LT estimates would be strongly correlated regardless of the analytical approach chosen.

Duplicate sample readings from the Lactate Plus analyzer were strongly related, however there was a small fixed bias, indicating that the values from the second sample were consistently lower than values from the first sample. In addition, there was a very small proportional bias. Both of these biases may be explained by using separate samples collected directly from the finger. The milking of the finger to obtain a blood sample can cause the dilution of the blood sample by interstitial fluid. The manufacturer warns the user against vigorous squeezing of the finger to obtain a blood drop. The use of a vasodilating cream may resolve this issue. When we used the same mixed blood sample as the reference analyzer, the proportional bias was eliminated, while the fixed bias was reduced by approximately 65%.

We also found that the portable analyzer was unable to analyze the blood sample 11% of the time, presumably from an insufficient sample volume. This was surprising given that the Lactate Plus lactate analyzer provides an audible signal to indicate when the test strip has a sufficient volume of blood for analysis. Our experience has shown that anticipating the filling of the test strip can result in both the audible signal and an error. However, even when great care is taken, one can still get an audible full signal and the error message.

Ridenour et al. advocated for a switch from fetal blood sampling to lactate analysis. [4] However, their data showed that the variability in blood [lactate] accounted for only 46% of the variability in pH. This could be due to the significant proportional bias that is apparent in their data (Ref 1, Figures 1 and 3). However, our analysis shows a fixed and proportional bias that are less than half reported by previous studies relying on Bland-Altman plots and simple comparison of means. [3 4] This suggests the modest correlation between fetal [lactate] and blood pH is best attributed to the independent regulation of blood lactate and pH rather than unreliable measurement of [lactate]. [27 28]

We did not compare the Lactate Plus lactate analyzer to known standards. This limits the precision with which we can quantify the accuracy of the portable analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. Our analysis assumes measurement error in both the portable and reference instrument. Thus it is likely that by comparing the Lactate Plus lactate analyzer directly to known lactate standards, our fixed bias would be reduced.

While some studies have used blood collected from trained athletes to compare portable lactate analyzers to bench top models, [5 6 8 10] several do not. [3-5 7 9] This seems quite appropriate given that the importance of accurate lactate measurement extends well beyond the athletic field. Our subjects were healthy and physically active, but not highly trained. This is unlikely to account for any difference between previous studies and ours given that we can find no reason to speculate that either lactate analyzer would more accurately measure [lactate] in one population compared to another.

Similarly, the choice of graded exercise protocol can affect lactate threshold determination. [29] Thus, our use of a personalized, discontinuous GXT likely produced LT values different from some other protocols. However, this would have no affect on our ability to accomplish the aims of our study, specifically to compare estimates of LT between lactate measurements produced by the portable and

reference analyzers.

In summary, the Lactate Plus analyzer is a valid and reliable instrument across a wide range of blood lactate concentrations. Any proportional or fixed bias in blood lactate concentration is nearly indistinguishable from zero. Therefore, the portable analyzer can be used to determine exercise intensities based on absolute or relative blood lactate concentrations. Sampling procedures can have a significant effect on the reliability of the portable analyzer, and the portable analyzer is prone to technical issues in nearly one out of ten samples.

CONTRIBUTORS

Sarah Hart collected and analyzed blood samples, and helped with data reduction.

Kathryn Drevets collected and analyzed blood samples, and helped with data reduction.

Micah Alford collected some data and performed statistical analysis.

Amanda Salacinski helped design the study, collected data, and revised the manuscript.

Brian E. Hunt designed the study, collected data, designed statistical analysis, and wrote the manuscript.

COMPETING INTERESTS

None

ETHICS APPROVAL

Ethics approval was provided by the Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study.

DATA SHARING STATEMEMNT

No additional data are available

ACKNOWLDGMENTS

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FIGURE LEGENDS

Figure 1. Determination of the Lactate threshold by visual inspection. Shown are data from a representative study participant and the lines of best fit that were determined independently for data from the YSI 2300 lactate analyzer and the Lactate Plus lactate analyzer. Blood samples could not be collected between stages 4 and 5. The Lactate Plus analyzer returned error message between stages 6 and 7.

Figure 2. Bland-Altman plot depicting the level of agreement between lactate concentrations determined by Lactate Plus portable analyzer the YSI bench top analyzer.

Figure 3. Ordinary least products regression analysis of the relation between lactate concentrations determined by the Lactate Plus portable analyzer and the YSI bench top analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equations and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 4. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the YSI bench top analyzer. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 5. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the Lactate Plus portable lactate analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

A method-comparison study regarding the validity and reliability of the Lactate Plus© analyzer

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SUMMARY

Article focus

- Determine the validity and reliability of the Lactate Plus analyzer and quantify any systematic bias.
- Determine the effect of any bias on the determination of lactate threshold
- Determine the effect that blood sampling methods have on validity and reliability

Key messages

- The Lactate Plus analyzer provides valid and reliable measurements of blood lactate concentration.
- The Lactate Plus analyzer demonstrates a small fixed and proportional bias.
- Sampling directly from the finger does increase the variability in measurement, likely due to the milking of the finger rather than the analyzer itself.

Strengths and limitations

- This study compares the accuracy and variability in measurements under both laboratory and field sampling conditions.
- We used least-product regression analysis to independently quantify fixed and proportional bias rather than Bland-Altman plots or least-squares regression, which lump these bias together or assumes there is no measurement error in the reference method.
- We did not compare either instrument to known lactate standards. This may limit our ability to precisely quantify the accuracy of the portable analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. This reduces the likelihood that our reference instrument is inaccurate or non-linear.

ABSTRACT

Objectives: The aims of this study were to: 1) determine the validity and reliability of the Nova Biomedical Lactate Plus portable analyzer, and quantify any fixed or proportional bias, 2) determine the effect of any bias on the determination of the lactate threshold, and 3) determine the effect that blood sampling methods have on validity and reliability. **Design and Participants:** In this method comparison study 15 active men and women performed a discontinuous graded exercise test to volitional exhaustion on a motorized treadmill. Blood samples were taken via finger prick and collected in micro capillary tubes for analysis by the reference instrument (Yellow Springs Instrument 2300 Glucose and Lactate Analyzer) at the end of each stage. Duplicate samples for the portable analyzer were either taken directly from the finger or from the micro capillary tubes. **Primary Outcome Measurements:** Ordinary least products regressions were used to assess validity, reliability, and bias in the portable analyzer. Lactate threshold was determined by visual inspection. **Results:** Though measurements from both instruments were correlated (r=0.91), the differences between instruments had large variability (SD = 1.45 $mM \cdot l^{-1}$) when blood was sampled directly from finger. This variability was reduced by ~95% when both instruments measured blood collected in the capillary tubes. As the proportional and fixed bias between instruments was small, there was no difference in estimates of the lactate threshold between instruments. Reliability for the portable instrument was strong (r=0.99, $p<0.05$) with no proportional bias (slope=1.02) and small fixed bias (-0.19 $mM \cdot l^{-1}$). **Conclusion:** The Lactate Plus analyzer provides accurate and reproducible measurements of blood lactate concentration that can be used to estimate workloads corresponding to blood lactate transitions or any absolute lactate concentrations.

INTRODUCTION

Not only is blood lactate accumulation a common measure in the physiological assessment of endurance athletes, but is also an important clinical measure.[1-4] Portable lactate analyzers have advantages over bench top models including: 1) their ability to rapidly sample blood lactate concentration ([lactate]), in or outside the laboratory, 2) they require a much smaller sample of blood (0.5 – 0.7µl) than many bench top analyzers (25 – 75µl), and 3) they can be purchased and operated at a lower cost than many bench top models.

Several studies have attempted to evaluate the validity and reliability of these portable analyzers.[3-10] While the majority of studies report the [lactate] measured using portable analyzers is similar to those of various bench top models, the mean difference between the reference and portable analyzer can be as much as 1.0 $mM \cdot l^{-1}$. This can represent nearly 10% of the full range of values in some populations. [11] This level of disagreement could be explained by the presence of systematic measurement error. Systematic measurement error can result in a proportional bias, where one instrument produces values that are different from those of another instrument by an amount that is proportional to the level of the measured variable, and/or a fixed bias, where one instrument gives values that are different from those of another instrument by a constant amount.[12 13] Thus, similar mean values between lactate analyzers could occur while the portable analyzer produces low values at lower [lactate] and high values at higher [lactate] or vice-versa. Previous studies have primarily relied on Bland-Altman analysis to determine the presence of any fixed bias. However, this approach does not allow the independent determination of bias, and thus has limited utility in assessing the presence of systematic measurement error. Therefore, while most data appear to show a substantial proportional and/or fixed bias the presence and degree of bias in portable lactate analyzers remains unresolved.[3 4 6-10] Furthermore, because previous studies have not directly examined these biases it is unclear if they are large enough to

affect estimates of various lactate parameters, such as pH or lactate threshold.

Blood sampling techniques may also affect measurement accuracy and reliability. Previous studies have either used intravenous blood drawn directly into a syringe,[3 7 9] or capillary blood from a finger stick drawn into capillary tubes then mixed as would be done in the laboratory.[6 10] Portable analyzers, however, are designed to sample blood directly from a puncture for ease of use in the field. When using a finger stick to draw blood it is not uncommon to require "milking" of the finger to get an adequate sample. This may dilute the lactate concentration by increasing interstitial fluid in the sample. It would seem important to understand and quantify the effect of differing blood-sampling procedures on the accuracy and reliability of these portable analyzers.

Given the questions that remain regarding the validity and reliability of portable lactate analyzers the specific aims of the present study were: 1) to determine the validity and reliability of the Lactate Plus analyzer (Nova Biomedical), and quantify any fixed and/or proportional bias, and 2) determine the effect that blood sampling methods have on validity and reliability.

METHODS

Fifteen young (20-36 yr.; mean = 24.5 yr.) men and women (6 women) participated in the study. All subjects reported at least 90 minutes of moderate to vigorous physical activity each week. All subjects read and signed an informed consent. The Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study. All procedures conformed to the Declaration of Helsinki.

Instruments

To determine the validity of the Lactate Plus analyzer we used the YSI 2300 Stat Plus Glucose

and Lactate analyzer from Yellow Springs Instruments (Yellow Springs, OH) as our reference instrument. This bench top laboratory analyzer uses a membrane-bound enzyme electrochemical methodology. L-lactate oxidase is immobilized in a thin membrane placed over an electrochemical probe. The enzyme catalyzes the conversion of L-lactate to pyruvate and hydrogen peroxide, the latter then being oxidized at the platinum anode to measure lactate concentration in whole blood or plasma. A new membrane was used for each data collection session. The analyzer was initially calibrated using a 5 $mM \cdot l^{-1}$, 15 $mM \cdot l^{-1}$, and 30 $mM \cdot l^{-1}$ solution. In addition, an automated quality control was performed in triplicate every 45 minutes using a 5 $mM \cdot l^{-1}$ solution. Blood samples were collected from a finger stick into two heparinized capillary tubes. Blood was then mixed in a micro centrifuge tube. Two 25µl samples were sequentially aspirated and measured by the analyzer.

The Lactate Plus analyzer uses an electrochemical lactate oxidase biosensor to measure lactate concentration in a 0.7 µl sample. Following the manufacturer instructions we used a low (1.0 – 1.6 $mM \cdot l^{-1}$) and high (4.0-5.4 $mM \cdot l^{-1}$) quality control solution to ensure the lactate analyzer was operating properly at the beginning of each data collection session. For the first nine participants three blood samples were taken directly from the finger between each stage of the graded exercise test (GXT). All samples were taken in this order: 1) portable directly from finger, 2) capillary tubes for the YSI 2300 from the finger, and 3) a second sample directly from finger using the portable analyzer. To assess the effect of blood sampling techniques on the accuracy of the portable analyzer blood was drawn from the finger into capillary tubes and allocated to both the YSI 2300 and portable analyzer for the last six participants.

Graded Exercise

Participants performed a discontinuous graded exercise test (GXT) on a motorized treadmill

(Quinton TM65). Each stage lasted two-minutes with a one-minute blood sampling period between stages. The finger was prepared for sampling just prior to the end of each exercise stage. During the 1-minute blood collection period participants straddled the treadmill belt while blood samples were taken from a finger. After one minute the participants resumed exercise at a higher speed or grade. The initial speed was 1.55 $m \cdot s^{-1}$ and 0% grade. The speed was increased either 0.50 or 0.67 $m \cdot s^{-1}$ for each stage until the participant's heart rate was at least 80% of their age-predicted maximum (220-age). After this point the speed remained constant while grade was increased 2.5% for each stage. Exercise continued until volitional exhaustion.

Data Analysis

Two methods were used to assess validity. First, a Bland-Altman plot was constructed to allow the reader to more directly compare our data to that of previous studies since this is the approach typically used. However, because fixed and proportional biases cannot be determined independently from these plots, ordinary least products regression analysis was used. Validity was determined from the correlation coefficient in combination with the presence and degree of bias. The degree of fixed bias was determined from the y-intercept 95% confidence intervals. If the confidence interval for the intercept includes the value of zero, then there is no fixed bias. Proportional bias was determined from the 95% confidence interval for the slope. If the confidence interval for the slope includes the value of 1.0, then there is no proportional bias. Ordinary least products regression gives different slopes and y-intercepts than does least squares regression because error is assumed in both portable and bench top analyzers. [12 13]

Lactate threshold (LT) was defined as the point at which blood [lactate] began to increase in a non-linear fashion.[14 15] The threshold was estimated by plotting [lactate] against GXT stage. These

graphs were visually inspected to determine the lines of best fit by the two evaluators. The following guidelines were used to help guide the evaluators: 1) at least 3 data points were included in each line, 2) both lines contained unique data points, and 3) lines were chosen that produced the highest R^2 with the smallest confidence intervals. Once the lines were chosen the equations for each line were set equal to one another and solved for the point of intersection (Figure 1). The values from each evaluator were averaged.[16] These equations were also used to calculate the stage that corresponded to an absolute blood [lactate] of 2.5 and 4.0 $mM \cdot l^{-1}$. A t-test for paired data was used to compare means between analyzers. A p-value of <0.05 was considered statistically significant.

Reliability was determined using ordinary least products regression to quantify the relationship between sequential measurements for both instruments.

RESULTS

Validity

Lactate values during graded exercise ranged from 1.2 to 16.4 $mM \cdot l^{-1}$. When both portable and bench top blood samples are each taken directly from the finger the mean difference between [lactate] measured by the portable analyzer and the bench top analyzer was small across the full range of lactate values as depicted in Figure 2. While the mean difference between the two instruments was near zero, differences between the instruments had a large variability (SD = 1.45 $mM \cdot l^{-1}$). Even though there can be large differences between values measured by the portable and bench top analyzer, the paired measurements were highly correlated as shown in Figure 3A. Least products regression indicated a small fixed bias (y-intercept = -0.28 $mM \cdot l^{-1}$) between [lactate] measured with the portable and bench top analyzers. There was no evidence of a proportional bias (95% CI = 0.94 to 1.15). When the same mixed blood sample was used by both analyzers, the fixed bias was reduced to -0.056 $mM \cdot l^{-1}$, while a small

proportional bias was evident (slope = 1.08) as shown in Figure 3B.

Regardless of blood sampling approach there was excellent agreement between estimates of the LT based on lactate values from the portable analyzer compared to those from the bench top analyzer (r = 0.97). Moreover, there was neither a proportional bias (95% CI for slope: 0.910 to 1.098), nor a fixed bias (95% CI for y-intercept: -0.396 to 0.325) in estimates of the lactate threshold from the portable analyzer. Given the lack of bias it is not surprising there was no difference between blood [La] at the LT (2.88_{NOVA}±0.53 vs. 3.15_{YSI}±0.46 $mM \cdot l^{-1}$; p=0.32). In addition the stages corresponding to absolute blood lactate values of 2.5 $mM \cdot l^{-1}$ (2.99_{NOVA} vs. 2.92_{YSI}) and 4.0 $mM \cdot l^{-1}$ (4.64_{NOVA} vs. 4.61_{YSI}) were not different between portable and bench top values (p = 0.86 for both).

Reliability

The relationship between duplicate measurements of [lactate] by the bench top analyzer was very strong (r=0.99, p<0.05). Ordinary least products regression indicated no proportional bias (slope = 0.99), and a small fixed bias (0.059 $mM \cdot l^{-1}$; Figure 4). Ordinary least products regression revealed a small proportional (slope = 1.20) and fixed bias (-0.54 $mM \cdot l^{-1}$; Figure 5A) when the two duplicate blood samples for the portable analyzer were taken directly from the finger. Thus, the reading from the second sample was typically lower than from the first. However, when two duplicate measurements were taken from the same mixed blood sample, their was no proportional bias (slope = 1.02) and the fixed bias was reduced to -0.19 $mM \cdot l^{-1}$).

A total of 242 blood samples were taken using the portable analyzer. Twenty-seven of these attempts resulted in error messages (E-4 – insufficient sample). Thus, about 1-in-10 measurement attempts resulted in errors.

DISCUSSION

There were three new findings in our study: 1) The very small proportional bias indicates that the Lactate Plus analyzer is a highly linear instrument, 2) multiple blood samples directly from the finger increases measurement error, and 3) the small proportional and fixed bias in the portable analyzer does not affect the ability to determine the lactate threshold.

We chose to use ordinary least products regression to characterize the relation between the Lactate Plus analyzer and our reference analyzer. Most studies have employed a combination of Bland-Altman plots and least squares regression to determine the degree of agreement between various portable analyzers and a corresponding reference analyzer.[3-10] The mean difference between analyzers, as determined through Bland-Altman plots, is determined by the interaction of any fixed and proportional bias. Therefore, the mean difference between methods does not solely reflect the accuracy or fixed bias of the device, but in some cases, the presence of a proportional bias or loss of linearity. The use of least squares regression to characterize the level of proportional bias, as reflected in the slope of the linear relation, is skewed because all error is assigned to the dependent variable, in this case the portable analyzer. The use of least products regression to compare methods avoids both of these issues, allowing independent and more accurate determination of any fixed or proportional bias.[12 13 17]

Numerous studies have compared blood lactate measured with various portable analyzers to several different bench top analyzers.[4 5 7-10] All have reported that these portable analyzers produce similar lactate values compared with their bench top counterparts with average differences ranging from -0.8 to 1.0 $mM \cdot l^{-1}$. However, differences of almost 1.0 $mM \cdot l^{-1}$ can significantly impact the use of absolute [lactate] to characterize training intensity or efficacy. Weltman et al. reported that women who trained at an intensity corresponding to about 2.5 $mM \cdot l^{-1}$ showed greater improvement in blood lactate

parameters, but less of an improvement in VO2max than did women training at their lactate threshold. [18] If true, then an error in the measurement of blood lactate concentration could lead to suboptimal improvements in either lactate parameters or VO2max. Of the two studies that have tested the Lactate Plus analyzer, only Tanner et al. [10] reported the absolute difference between this portable analyzer and a reference analyzer (-0.8 $mM \cdot l^{-1}$). Our data show a much smaller difference between the Lactate Plus analyzer and the YSI bench top analyzer (fixed bias = -0.056 $mM \cdot l^{-1}$). Though not specifically assessed, it does appear that Tanner's reported difference between the hand held and reference analyzer is significantly influenced by a proportional bias (Ref 8, Figures 4 and 5). The fact that our data shows little proportional bias (Figure 3) may account for the greater agreement between analyzers that we observed. It is possible that if Tanner had been able to independently determine the proportional and fixed bias, their analysis may have revealed a small bias similar to ours. Differences in reference instruments would not likely explain the greater measurement error reported by Tanner, given that their instrument undergoes a 3-point calibration and 2-point calibration check every few hours, similar to our reference instrument.

Given that we found a very small proportional bias the estimation of the LT from [lactate] measured by the Lactate Plus analyzer agreed very well with those determined from [lactate] measured by the reference analyzer. Moreover, given the small fixed bias, it was not surprising that the lactate values from the portable analyzer provided similar estimates of the workload corresponding to the 2.5 $mM \cdot l^{-1}$ and the 4.0 $mM \cdot l^{-1}$ absolute lactate concentrations. These lactate concentrations were chosen because they have both sport and clinical significance. [1 2 19 20] The strong correlation coefficient and small biases suggest that the Lactate Plus analyzer can be used to accurately determine exercise intensities based on any blood lactate parameter.

Determination of the LT by visual inspection has come under scrutiny. [21 22] To reduce

subjectivity our approach to visual inspection is guided by several principles similar to those used by others. [16 23] Several methods of assessing the LT have been proposed that purport to be more objective. [14 16 24] However, many of these methods are known to be significantly affected by data outliers and/or missing data. [25 26] Therefore, the choice of any analytical approach has a subjective component. While our approach likely produces LT values that are different from other approaches, it produced values consistent with other studies that employed similar approaches to LT estimation. [18 23] When one considers the strong correlation and small biases in our data, it seems likely the LT estimates would be strongly correlated regardless of the analytical approach chosen.

Duplicate sample readings from the Lactate Plus analyzer were strongly related, however there was a small fixed bias, indicating that the values from the second sample were consistently lower than values from the first sample. In addition, there was a very small proportional bias. Both of these biases may be explained by using separate samples collected directly from the finger. The milking of the finger to obtain a blood sample can cause the dilution of the blood sample by interstitial fluid. The manufacturer warns the user against vigorous squeezing of the finger to obtain a blood drop. The use of a vasodilating cream may resolve this issue. When we used the same mixed blood sample as the reference analyzer, the proportional bias was eliminated, while the fixed bias was reduced by approximately 65%.

We also found that the portable analyzer was unable to analyze the blood sample 11% of the time, presumably from an insufficient sample volume. This was surprising given that the Lactate Plus lactate analyzer provides an audible signal to indicate when the test strip has a sufficient volume of blood for analysis. Our experience has shown that anticipating the filling of the test strip can result in both the audible signal and an error. However, even when great care is taken, one can still get an audible full signal and the error message.

Ridenour et al. advocated for a switch from fetal blood sampling to lactate analysis. [4] However, their data showed that the variability in blood [lactate] accounted for only 46% of the variability in pH. This could be due to the significant proportional bias that is apparent in their data (Ref 1, Figures 1 and 3). However, our analysis shows a fixed and proportional bias that are less than half reported by previous studies relying on Bland-Altman plots and simple comparison of means. [3 4] This suggests the modest correlation between fetal [lactate] and blood pH is best attributed to the independent regulation of blood lactate and pH rather than unreliable measurement of [lactate]. [27 28]

We did not compare the Lactate Plus lactate analyzer to known standards. This limits the precision with which we can quantify the accuracy of the portable analyzer. However, our reference instrument was calibrated using 3 known lactate standards across a supraphysiologic range. Our analysis assumes measurement error in both the portable and reference instrument. Thus it is likely that by comparing the Lactate Plus lactate analyzer directly to known lactate standards, our fixed bias would be reduced.

While some studies have used blood collected from trained athletes to compare portable lactate analyzers to bench top models, [5 6 8 10] several do not. [3-5 7 9] This seems quite appropriate given that the importance of accurate lactate measurement extends well beyond the athletic field. Our subjects were healthy and physically active, but not highly trained. This is unlikely to account for any difference between previous studies and ours given that we can find no reason to speculate that either lactate analyzer would more accurately measure [lactate] in one population compared to another.

Similarly, the choice of graded exercise protocol can affect lactate threshold determination. [29] Thus, our use of a personalized, discontinuous GXT likely produced LT values different from some other protocols. However, this would have no affect on our ability to accomplish the aims of our study, specifically to compare estimates of LT between lactate measurements produced by the portable and

reference analyzers.

In summary, the Lactate Plus analyzer is a valid and reliable instrument across a wide range of blood lactate concentrations. Any proportional or fixed bias in blood lactate concentration is nearly indistinguishable from zero. Therefore, the portable analyzer can be used to determine exercise intensities based on absolute or relative blood lactate concentrations. Sampling procedures can have a significant effect on the reliability of the portable analyzer, and the portable analyzer is prone to technical issues in nearly one out of ten samples.

CONTRIBUTORS

Sarah Hart collected and analyzed blood samples, and helped with data reduction.

Kathryn Drevets collected and analyzed blood samples, and helped with data reduction.

Micah Alford collected some data and performed statistical analysis.

Amanda Salacinski helped design the study, collected data, and revised the manuscript.

Brian E. Hunt designed the study, collected data, designed statistical analysis, and wrote the manuscript.

COMPETING INTERESTS

None

ETHICS APPROVAL

Ethics approval was provided by the Institutional Review Boards at Wheaton College and the Northern Illinois University approved this study.

DATA SHARING STATEMEMNT

No additional data are available

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Lactate test strips and quality assurance solutions for the Lactate Plus lactate analyzer were provided by Lactate.com.

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FIGURE LEGENDS

Figure 1. Determination of the Lactate threshold by visual inspection. Shown are data from a representative study participant and the lines of best fit that were determined independently for data from the YSI 2300 lactate analyzer and the Lactate Plus lactate analyzer. Blood samples could not be collected between stages 4 and 5. The Lactate Plus analyzer returned error message between stages 6 and 7.

Figure 2. Bland-Altman plot depicting the level of agreement between lactate concentrations determined by Lactate Plus portable analyzer the YSI bench top analyzer.

Figure 3. Ordinary least products regression analysis of the relation between lactate concentrations determined by the Lactate Plus portable analyzer and the YSI bench top analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equations and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 4. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the YSI bench top analyzer. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 5. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the Lactate Plus portable lactate analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.

Figure 1

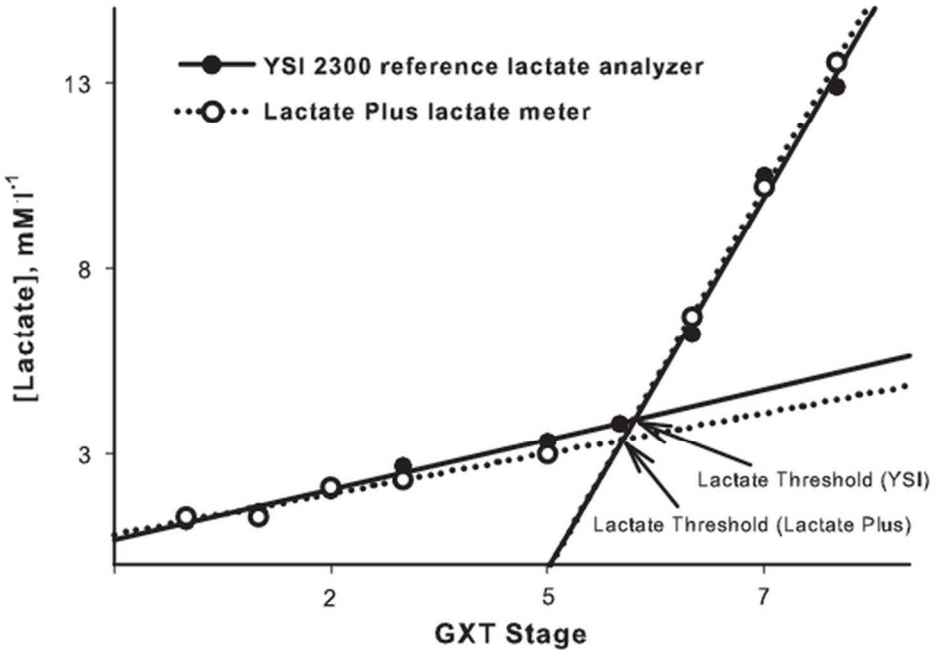

Figure 1. Determination of the Lactate threshold by visual inspection. Shown are data from a representative study participant and the lines of best fit that were determined independently for data from the YSI 2300 lactate analyzer and the Lactate Plus lactate meter.
90x95mm (300 x 300 DPI)

Figure 2

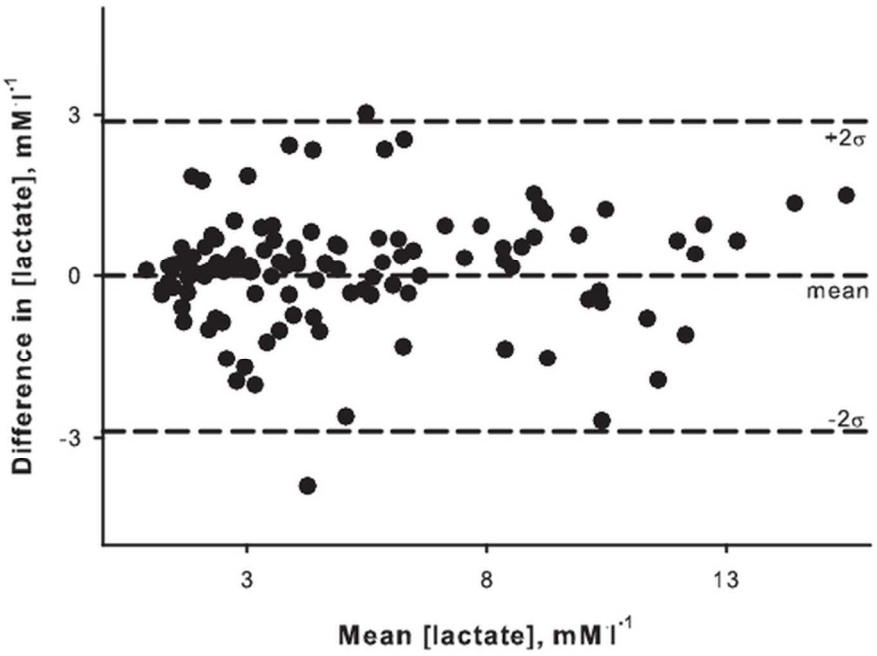

Figure 2. Bland-Altman plot depicting the level of agreement between lactate concentrations determined by Lactate Plus hand-held analyzer the YSI bench top analyzer.
90x97mm (300 x 300 DPI)

Figure 3

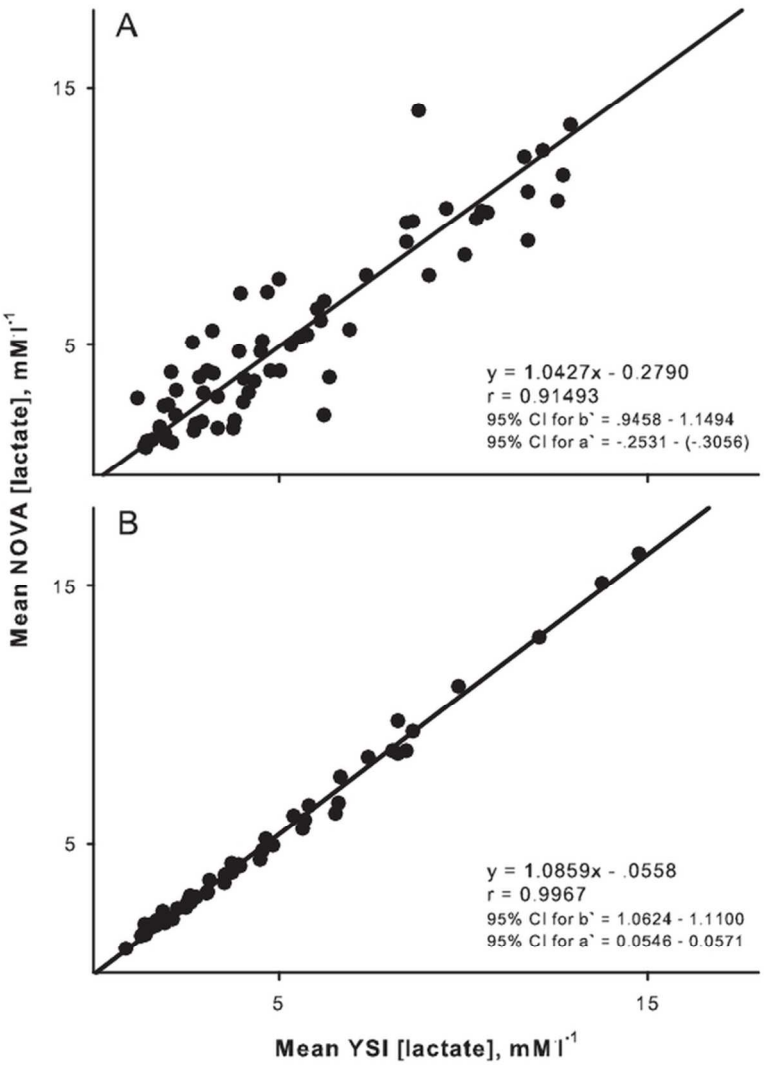

Figure 3. Ordinary least products regression analysis of the relation between lactate concentrations determined by the Lactate Plus hand-held analyzer and the YSI bench top analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equations and confidence intervals for slope (b) and y-intercept (a) are presented.
90x124mm (300 x 300 DPI)

Figure 4

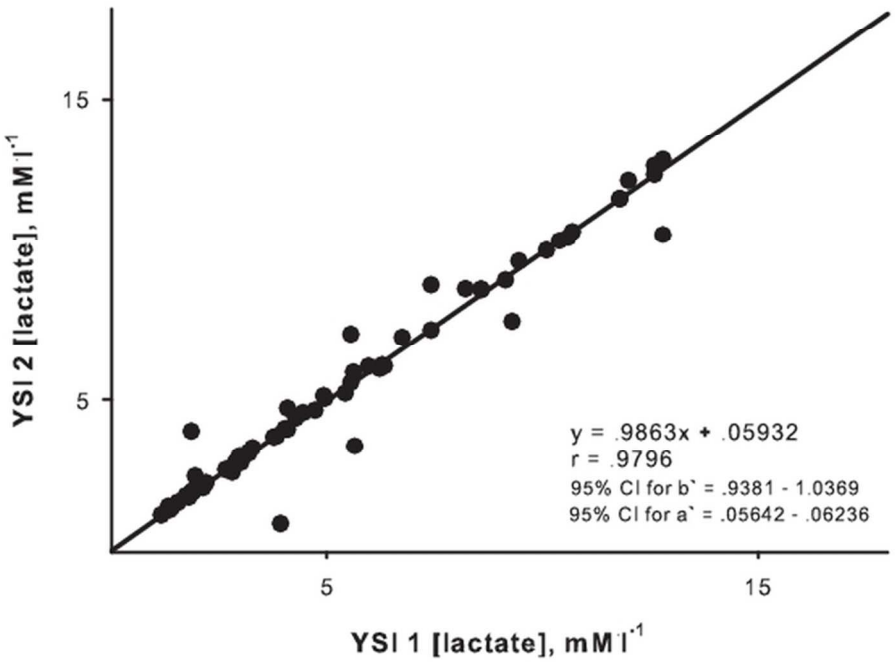

Figure 4. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the YSI bench top analyzer. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.
90x94mm (300 x 300 DPI)

Figure 5

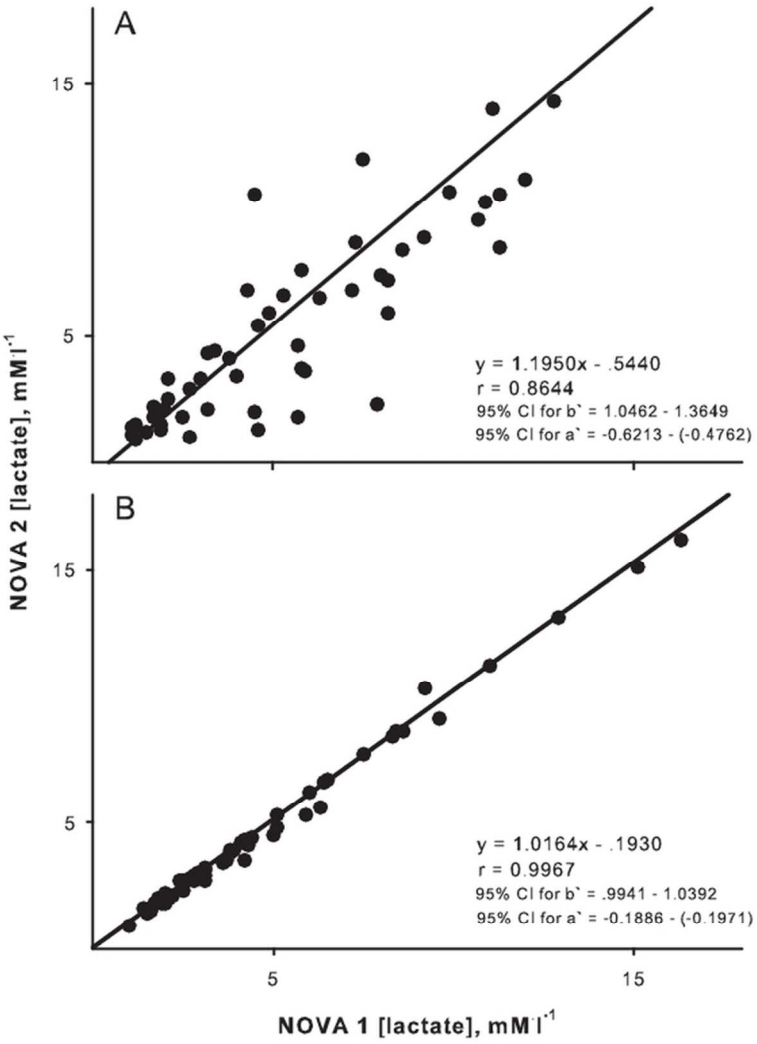

Figure 5. Ordinary least products regression analysis of the relation between sequential estimates of blood lactate concentration by the Lactate Plus hand-held lactate analyzer. Panel A – when separate samples for each analyzer were collected directly from finger. Panel B – when a common sample of blood was used by both analyzers. Regression equation and confidence intervals for slope (b) and y-intercept (a) are presented.
90x122mm (300 x 300 DPI)